INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : C07K 5/06, A61K 38/05	**A1**	(11) International Publication Number: **WO 96/11209**
		(43) International Publication Date: 18 April 1996 (18.04.96)

(21) International Application Number: PCT/GB95/02362

(22) International Filing Date: 5 October 1995 (05.10.95)

(30) Priority Data:
9420057.3	5 October 1994 (05.10.94)	GB
9504907.8	10 March 1995 (10.03.95)	GB
9509431.4	10 May 1995 (10.05.95)	GB

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(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

(54) Title: PEPTIDYL COMPOUNDS AND THEIR THERAPEUTIC USE AS INHIBITORS OF METALLOPROTEASES

(57) Abstract

Compounds of general formula (I) have utility as inhibitors of matrix metalloproteinases and TNF.

$$R^7S{-}\overset{R^8}{\underset{}{C}}{-}C(O){-}NH{-}\overset{R^1}{\underset{}{C^*}}{-}C(O){-}N(R^2){-}\overset{R^3}{\underset{}{C^*}}{-}C(O){-}X \qquad (I)$$

Peptidyl compounds and their therapeutic use as inhibitors of metalloproteases

Field of the Invention

This invention relates to a novel class of peptidyl derivatives, to processes for their

5   preparation, and to their use in medicine.

Background to the Invention

In normal tissues, cellular connective tissue synthesis is offset by extracellular matrix

degradation, the two opposing effects existing in dynamic equilibrium. Degradation

of the matrix is brought about by the action of proteinases released from resident

10   connective tissue cells and invading inflammatory cells, and is due, in part, to the

activity of at least three groups of metalloproteinases. These are the collagenases

(interstitial collagenase, MMP-1; PMN collagenase, MMP-8, collagenase-3,

MMP13), the gelatinases (gelatinase A, MMP-2, 72kDa-gelatinase, Type IV

collagenase; gelatinase B, MMP-9, 92kDa-gelatinase, Type IV collagenase) and the

15   stromelysins (proteoglycanase, MMP-3, stromelysin-1, transin; stromelysin-2,

MMP:10; stromelysin-3, MMP:11). Normally these catabolic enzymes are tightly

regulated at the level of their synthesis and secretion and also at the level of their

extracellular activity, the latter through the action of specific inhibitors, such as

TIMP (tissue inhibitors of metalloproteinase), which form inactive complexes with

20   metalloproteinases, and more general proteinase inhibitors such as a_2 -

macroglobulins.

The accelerated, uncontrolled breakdown of connective tissues by metalloproteinase

catalysed resorption of the extracellular matrix is a feature of many pathological

conditions such as rheumatoid arthritis, osteoarthritis, septic arthritis, corneal,

25   epidermal or gastric ulceration; tumour metastasis or invasion; periodontal disease,

proteinuria, coronary thrombosis associated with atherosclerotic plaque rupture and

bone disease. The inhibitors claimed herein may also be useful in preventing the

pathological squaelae following a traumatic injury that could lead to a permanent

disability. These compounds may also have utility as a means for birth control by

30   preventing ovulation or implantation. It can be expected that the pathogenesis of

such diseases is likely to be modified in a beneficial manner by the administration

of metalloproteinase inhibitors and numerous compounds have been suggested for

this purpose [for a general review see R C Wahl, *et al* Ann. Rep, Med. Chem. <u>25</u>: 175-184, Academic Press Inc., San Diego (1990)].

A number of small peptide like compounds which inhibit metalloproteinases have been described. Perhaps the most notable of these are those relating to angiotensin converting enzyme (ACE) where such agents act to block the conversion of the decapeptide angiotensin I to angiotensin II, a potent pressor substance. Compounds of this type are described in EP-A-0012401. Also, related mercaptoamide peptidyl derivatives have shown ACE inhibitor activity *in vitro* and *in vivo* (H N Weller *et al* (1984), Biochem Biophys. Res. Comm., <u>125</u> (1):82-89).

TNFα is a cytokine which is produced initially as a cell-associated 28kD precursor. It is released as an active, 17kD form (D-M Jue *et al*, (1990) Biochemistry, <u>29</u>:8371-8377), which can mediate a large number of deleterious effects *in vivo*. When administered to animals or humans it causes inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration can also cause cachexia and anorexia. Accumulation of excessive TNFα can be lethal. There is considerable evidence from animal model studies that blocking the effects of TNFα with specific antibodies can be beneficial in acute infections, shock states, graft versus host reactions and autoimmune disease. TNFα is also an autocrine growth factor for some myelomas and lymphomas and can act to inhibit normal heamatopoiesis in patients with these tumours.

Preventing the production or action of TNFα is, therefore, predicted to be a potent therapeutic strategy for many inflammatory, infectious, immunological or malignant diseases. These include, but are not restricted to, septic shock, haemodynamic shock and sepsis syndrome (Mathison et al (1988) J. Clin. Invest. <u>81</u>:1925-1937; Miethke et al (1992), J. Exp. Med. <u>175</u>:91-98), post-ischaemic reperfusion injury, malaria (Grau et al (1989), Immunol. Rev. <u>112</u>:49-70); mycobacterial infection (Barnes et al (1992) Infect. Imm. <u>60</u>:1441-6), meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, rheumatoid arthritis, multiple sclerosis, radiation damage, toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1 and hyperoxic alveolar injury.

Current clinical anti-TNFα strategies involve the use of corticosteroids such as dexamethasone, and the use of cyclosporin-A or FK506, which are non-specific inhibitors of cytokine gene transcription. Phosphodiesterase inhibitors such as pentoxyfilline have been shown to be more specific inhibitors of TNFα gene

5   transcription (Endres S. (1991) Immunol. 72:56-60, Schandene et al (1992), Immunol. 76:30-34, Alegre ML, et al (1991); Transplantation 52:674-679, Bianco et al (1991) Blood 78:1205-1221). Thalidomide has also been shown to inhibit TNFα production by leucocytes (Sampajo et al (1991), J. Exp. Med. 173:699-703). In experimental settings, anti-TNFα monoclonal antibodies, soluble TNF receptors

10 and soluble TNF receptor/immunoadhesins have been shown to specifically inhibit the effects of TNFα action (Bagby et al (1991) J. Infect. Dis. 163:83-88, Charpentier et al. (1991) Presse-med. 20:2009-2011, Silva et al (1990) J. Infect. Dis. 162:421-427; Franks et al (1991) Infect. Immun. 59:2609-2614, Tracey et al (1987) Nature 330:662-664; Fischer et al (1992) PNAS USA in press, Lesslauer et

15 al (1991) Eur. J. Immunol. 21:2883-2886, Ashkenazi et al (1991) PNAS USA 88:10535-10539).

It has recently been shown that the effects of TNF are mediated by two peptides, TNFα and TNFβ. Although these peptides have only 30% homology with each other, they activate the same receptors and are encoded by immediately adjacent

20 genes. As used herein, the term tumour necrosis factor or TNF therefore means tumour necrosis factor α and peptides having a high degrees of sequence homology with, or substantially similar physiological effects to, TNFα, for example TNFβ.

One of the objectives of the present invention is to provide compounds which substantially inhibit the release of TNF from cells, and therefore may be used in the

25 treatment of conditions mediated by TNF. Such uses include, but are not limited to, the treatment of inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.

Compounds which have the property of inhibiting the action of metalloproteinases

30 involved in connective tissue breakdown such as collagenase, stromelysin and gelatinase have been shown to inhibit the release of TNF both in vitro and in vivo (AJH Gearing et al (1994), Nature, 370:555-557; GM McGeehan et al (1994),

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Nature, 370:558-561: MJ Crimmin *et al*, WO 93/20047). All of these reported inhibitors contain a hydroxamic acid zinc binding group.

It is, therefore, a further objective of this invention to provide compounds which, in addition to inhibiting TNF release, also inhibit the action of MMPs, and hence

5   may be used in the treatment of patients who suffer from conditions mediated by TNF and/or MMPs.

As appreciated by those of skill in the art the significant proportion of homology between human fibroblast collagenase, stromelysin and gelatinase leads to the possibility that a compound that inhibits one enzyme may to some degree inhibit all

10   of them.

Compounds that inhibit collagenase, which possess structural portions akin to those of the instant invention include those encompassed by U.S.4,511,504 issued Apr. 16, 1985; U.S. 4,568,666, issued Feb 4, 1986.

Compounds of related structure that are claimed to inhibit stromelysin

15   (proteoglycanase) are encompassed by U.S.4,771,037, issued Sept. 13, 1988.

The applicants believe that stromelysin and collagenase inhibitors have utility in preventing articular cartilage damage associated with septic arthritis. Bacterial infections of the joints can elicit an inflammatory response that may then be perpetuated beyond what is needed for removal of the infective agent resulting in

20   permanent damage to structural components. Bacterial agents have been used in animal models to elicit an arthritic response with the appearance of proteolytic activities. See J. P. Case *et al* (1989), J. Clin. Invest., 84:1731-40; R. J. Williams *et al* (1990), Arth. Rheum., 33: 533-41.

The applicants also believe that inhibitors of stromelysin, collagenase and gelatinase

25   will be useful to control tumour metastasis, optionally in combination with current chemotherapy and/or radiation. See L. M. Matrisian *et al* (1986), Proc. Natl. Acad. Sci., USA, 83:9413-7; S. M. Wilhelm *et al* (1987), Ibid. 84:6725-29; Z. Werb *et al* (1989), J. Cell Biol., 109:872-889; L. A. Liotta *et al* (1983), Lab. Invest., 49:636-649; R. Reich *et al* in Metatasis; Ciba Foundation Symposium,

30   Wiley, Chicester, 1988, pp. 193-210.

Secreted proteinases such as stromelysin, collagenase and gelatinase play an important role in processes involved in the movement of cells during metastasic

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tumour invasion. Indeed, there is also evidence that the matrix metalloproteinases are overexpressed in certain metastatic tumour cell lines. In this context, the enzyme functions to penetrate underlying basement membranes and allow the tumour cell to escape from the site of primary tumour formation and enter the circulation. After adhering to blood vessel walls, the tumour cells use these same metalloproteinases to pierce underlying basement membranes and penetrate other tissues, thereby leading to tumour metastasis. Inhibition of this process would prevent metastasis and improve the efficacy of current treatments with chemotherapeutics and/or radiation. These inhibitors should also be useful for controlling periodontal diseases, such as gingivitis. Both collagenase and stromelysin activities have been isolated from fibroblasts derived from inflamed gingiva (V. J. Uitto *et al* (1981), J.Periodontal Res., 16:417-424). Enzyme levels have been correlated to the severity of gum disease; C. M. Overall *et al* (1987), J. Periodontal Res., 22:81-88.

Proteolytic processes have also been observed in the ulceration of the cornea following alkali burns (S. I. Brown *et al* (1969), Arch. Opthalmol., 81:370-373). Mercapto-containing peptides do inhibit the collagenase isolated from alkali-burned rabbit cornea (F. R. Burns *et al* (1989), Invest. Opthalmol, 30:1569-1575). Treatment of alkali-burned eyes or eyes exhibiting corneal ulceration as a result of infection with inhibitors of these metalloendoproteinases in combination with sodium citrate or sodium ascorbate and/or antimicrobials may be effective in preventing developing corneal degradation.

Stromelysin has been implicated in the degradation of structural components of the glomerular basement membrane (GBM) of the kidney, the major function of which is to restrict passage of plasma proteins into the urine (W. H. Baricos *et al* (1989), Biochem. J., 254:609-612). Proteinuria, a result of glomerular disease, is excess protein in the urine caused by increased permeability of the GBM to plasma proteins. The underlying causes of the increased GBM permeability are unknown, but proteinases including stromelysin may play an important role in glomerular diseases. Inhibition of this enzyme may alleviate the proteinura associated with kidney malfunction.

It is suggested that inhibition of stromelysin activity may prevent the rupturing of atherosclerotic plaques leading to coronary thrombosis. The tearing or rupture of

atherosclerotic plaques is the most common event initiating coronary thrombosis. Destabilisation and degradation of the connective tissue matrix surrounding these plaques by proteolytic enzymes or cytokines released by infiltrating inflammatory cells has been proposed as a cause of plaque fissuring. Such tearing of these plaques can cause an acute thrombolytic event as blood rapidly flows out of the blood vessel. High levels of stromelysin RNA message have been found to be localised to individual cells in atherosclerotic plaques removed from heart transplant patients at the time of surgery (A. M. Henney *et al* (1991), Proc. Nat'l. Acad. Sci. USA, 88:8154-8158). Inhibition of stromelysin by these compounds may aid in preventing or delaying the degradation of the connective tissue matrix that stabilises the atherosclerotic plaques, thereby preventing events leading to acute coronary thrombosis.

It is also believed that specific inhibitors of stromelysin and collagenase should be useful as birth control agents. There is evidence that expression of metalloproteinases, including stromelysin and collagenase, is observed in unfertilised eggs and zygotes and at further cleavage stages and increased at the blastocyst stage of fetal development and with endoderm differentiation (C. A. Brenner *et al* (1989), Genes & Develop., 3:848-59). By analogy to tumour invasion, a blastocyst may express metalloproteinases in order to penetrate the extracelluar matrix of the uterine wall during implantation. Inhibition of stromelysin and collagenase during these early development processes should presumably prevent normal embryonic development and/or implantation in the uterus. Such intervention would constitute a novel method of birth control. In addition there is evidence that collagenase is important in ovulation processes. In this example, a covering of collagen over the apical region of the follicle must be penetrated in order for the ovum to escape. Collagenase has been detected during this process and an inhibitor has been shown to be effective in preventing ovulation (J. F. Woessner *et al* (1989), Steroids, 54:491-499). There may also be a role for stromelysin activity during ovulation (C. K. L. Too *et al* (1984), Endocrin., 115:1043-1050).

Collagenolytic and stromelysin activity have also been observed in dystrophic epidermolysis bullosa (A. Kronberger *et al* (1982), J. Invest. Dermatol., 79:208-211; D. Sawamura *et al* (1991), Biochem. Biophys. Res. Commun., 184:1003-8).

Inhibition of metalloendoproteinases should limit the rapid destruction of connective components of the skin.

In addition to extracelluar matrix comprising structural components, stromelysin can degrade other *in vivo* substrates including the inhibitors a_1-proteinase inhibitor and

5   may therefore influence the activities of other proteinases such as elastase (P. G. Winyard *et al* (1991), FEBS Letts., 279,1:91-94). Inhibition of the matrix metalloendoproteinases may potentiate the antiproteinase activity of these endogenous inhibitors.

From recent publications it is evident that several new enzymes of the MMP family

10   have been identified, some of which maybe important in disease. Collagenase 3, an enzyme unique to breast carcinoma cells may have utility in breast cancer (JMP Freije *et al* (1994), J. Biol. Chem., 269 (24): 16766-16773), whilst MT-MMP, another member of the MMP family has been shown to be a key enzyme in the activation of gelatinase A (H Sato *et al* (1994), Nature, 370:61-65). Gelatinase A

15   is an important enzyme in the growth and metastasis of tumours (such as defined above).

The degradation of β-Amyloid Precusor Protein (APP) has been shown to generate amyloid plaques, a major constituent of the senile plaques, found in patients with Alzheimers Disease (AD). Two recent publications have identified metalloproteinase

20   enzymes that cleave APP to the amyloid plaque (CR Abraham *et al* (1994), Biochemistry, 33:192-199; G Huber *et al* (1994), Biochem. Biophys. Res. Comm., 201 (1):45-53).

As appreciated by those of skill in the art, the significant proportion of homology between these new enzymes and other MMPs leads to the possibility that a

25   compound that inhibits one enzyme may to some degree inhibit these new enzymes. Therefore, inhibitors encompassed in this invention may be useful in the diseases in which these new enzymes are implicated.

SUMMARY OF THE INVENTION

The invention encompasses novel mercaptoalkylpeptidyl compounds of formula (I)

30   which are useful inhibitors of matrix metalloproteinases and/or TNFα mediated diseases including degenerative diseases (such as defined above) and certain cancers.

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In a first aspect of the invention there is provided a compound of general formula (I):

(I)

Wherein:

R^1 is C_{1-6} alkyl, C_{2-6} alkenyl, -C_{1-6} alkyl-aryl, aryl, -C_{1-6} alkyl-heteroaryl, heteroaryl or -C_{1-6} alkyl-AR^9 where A represents O,NR^9 or S(O)$_m$ where m=0-2, and R^9 is H, C_{1-4} alkyl, aryl, heteroaryl, -C_{1-4} alkyl-aryl or -C_{1-4} alkyl-heteroaryl; if A=NR^9 the groups R^9 may be the same or different;

R^2 is H or C_{1-6} alkyl;

R^3 is [Alk]$_n$$R^6$ where Alk is C_{1-6} alkyl or C_{2-6} alkenyl and n is zero or 1;

X is N$R^4$$R^5$ where either R^4 is hydrogen or C_{1-6} alkyl optionally substituted by amino (NH$_2$), aryl, arylamino, protected amino, di(C_{1-6} alkyl)amino, mono(C_{1-6} alkyl)amino, CO$_2$H, protected carboxyl, carbamoyl, mono(C_{1-6} alkyl)carbamoyl or di(C_{1-6} alkyl)carbamoyl, and R^5 is hydrogen or C_{1-6} alkyl; or N$R^4$$R^5$ forms a ring such as pyrrolidino, piperidino or morpholino;

R^7 is hydrogen or R^{10}CO where R^{10} is C_{1-4} alkyl, -C_{1-4} alkyl-aryl, -C_{1-4} alkyl-heteroaryl, cyclo(C_{3-6})alkyl, -C_{1-4} alkyl-cyclo(C_{3-6})alkyl, C_{2-6} alkenyl, -C_{2-6} alkenyl-aryl, aryl or heteroaryl;

R^8 is aryl (substituted with R^{11}), heteroaryl (optionally substituted with R^{11}), C_{1-4} alkyl-R^{11}, -C_{1-4} alkyl-aryl (substituted with R^{11}), -C_{1-4} alkyl-heteroaryl (optionally substituted with R^{11}), cyclo(C_{3-6})alkyl (optionally substituted with R^{11}), cyclo(C_{3-6})alkenyl (optionally substituted with R^{11}), -C_{1-4} alkyl-cyclo(C_{3-6})alkyl (optionally substituted with R^{11}), or any of the three groups

C_{0-4} alkyl $-\left[\begin{array}{c}\\ \overset{}{\underset{\underset{R^{12}}{N}}{}}\end{array}\right]_p$ or

C_{0-4} alkyl $-\left[\begin{array}{c}\\ \overset{O}{\underset{\underset{R^9}{N}}{}}\end{array}\right]_p$

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C_{0-4} alkyl $-\left[\begin{array}{c} B \\ \overset{}{\underset{C}{}} =O\end{array}\right]$

where p is 1 or 2 and B and C are independently selected from O, S, $C(R^9)_2$ and NR^9;

10   R^6 is AR^9, cyclo(C_{3-6})alkyl, cyclo(C_{3-6})alkenyl, C_{1-6} alkyl, -C_{1-6} alkoxy-aryl, benzyloxyaryl, aryl, heteroaryl, -C_{1-3} alkyl-heteroaryl, -C_{1-3} alkyl-aryl, -C_{1-6} alkyl-$COOR^9$, -C_{1-6} alkyl-NHR, CONHR, $NHCO_2R$, $NHSO_2R$ or NHCOR, R being defined as for R^{10};

          R^{11} is SO_2R^{13}, SR^7, SR^9, COR^{13}, $N(R^9)_2$, NR^9R^{12}, OR^9, succinimido or
15   phthalimido;

          R^{12} is H or COR^9, CO_2R^9 (where R^9 is not H), $CONHR^9$ or SO_2R^9 (where R^9 is not H); and

          R^{13} is OH, OC_{1-4} alkyl, O-C_{1-4} alkyl-aryl, $N(R^9)_2$ (in which the R^9s are the same or different), C_{1-4} alkyl, aryl, heteroaryl, -C_{1-4} alkyl-aryl or -C_{1-4} alkyl-
20   heteroaryl;

the compound being in the form of a non-salt, salt, solvate or hydrate.

Preferred compounds of the invention include those in which, independently or in any combination have:

R^1 is C_{1-6} alkyl or C_{1-4} alkylAR^9 where A is $S(O)_m$,NR^9, or O and m=0,1 or 2, and R^9 is H, C_{1-4} alkyl or aryl;

R^2 is H or C_{1-4} alkyl;

R^3 is $[Alk]_nR^6$ where n=0 or 1, Alk is C_{1-4} alkyl and R^6 is C_{1-4} alkyl, C_{1-3} alkylaryl, C_{1-3} alkylheteroaryl or AR^9;

R^4 is H;

R^5 is H or C_{1-6} alkyl;

NR^{4}R^5 may form a 5-7 membered ring such as a pyrrolidine, piperidine or morpholine;

R^7 is H or R^{10}CO where R^{10} is C_{1-4} alkyl;

R^8 is C_{1-4} alkylR^{11}, C_{1-4} alkenylR^{11}, Cyclo(C_{3-6})alkylR^{11};

R^{11} is COR^{13}, NR^{9}R^{12}, N(R^9)$_2$, succinimido or phthalimido,

R^{12} is COR^9, CO2R^9 (provided R9 is not H), or SO$_2$R^9(provided R9 is not H); and R^{13} is OH, OC$_{1-4}$ alkyl or N(R^9)$_2$;

Compounds of the invention have IC$_{50}$ values below 50mM against the MMP enzymes and/or below 50mM in the whole cell assay of TNF inhibition.

It will be appreciated that the compounds according to the invention can contain one or more asymmetrically substituted carbon atoms, for example those marked with an asterisk in formula (1). The presence of one or more of these asymmetric centres in a compound of formula (1) can give rise to stereoisomers, and in each case the invention is to be understood to extend to all such stereoisomers, including enantiomers and diastereomers, and mixtures including racemic mixtures thereof.

In the formulae herein, the ~ line is used at a potential asymmetric centre to represent the possibility of R- and S- configurations, the < line and the ...... line to represent a unique configuration at an asymmetric centre.

As used in this specification, alone or in combination, the term "C_{1-6} alkyl" refers to a straight or branched chain alkyl moiety having from one to six carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, t-butyl, pentyl, hexyl and the like.

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The term "C_{1-4} alkyl" refers to a straight or branched chain alkyl moiety having from one to four carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, t-butyl and the like.

The term "C_{2-6} alkenyl" refers to a straight or branched chain alkyl moiety having two to six carbon atoms and having in addition one double bond, of either E or Z stereochemistry where applicable. This term would include for example, vinyl, 1-propenyl, 1- and 2- butenyl, 2- methyl-2-propenyl etc.

The term "cyclo (C_{3-6}) alkyl" refers to a saturated alicyclic moiety having from three to six carbon atoms and includes for example cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

The term "cyclo (C_{3-6}) alkenyl" refers to an alicyclic moiety having from three to six carbon atoms and having in addition one double bond. This term would include for example cyclopentenenyl or cyclohexenyl.

There term "aryl" means an optionally substituted phenyl or naphthyl group with the substituent(s) being selected, for example, from halogen, trifluoromethyl, C_{1-6} alkyl, alkoxy, phenyl and the like. The term "halogen" means fluorine, chlorine, bromine or iodine.

The terms "protected amino" and "protected carboxy" mean amino and carboxy groups which are protected in a manner familiar to those skilled in the art. For example, an amino group can be protected by a benzyloxycarbonyl, *tert*-butoxycarbonyl, acetyl or like groups, or in the form of a phthalimido or like group. A carboxyl group can be protected in the form of a readily cleavable ester such as the methyl, ethyl, benzyl or *tert*-butyl ester.

The term "alkoxy" refers to a straight chain or branched chain alkoxy group containing a maximum of six carbon atoms, such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, *tert*-butoxy and the like.

The term "C_{0-4} alkyl" refers to a bond or straight or branched chain alkyl moiety having from up to four carbon atoms, including for example, methyl, ethyl, propyl, isopropyl and the like.

The term "heteroaryl" refers to aromatic ring systems of five to ten atoms of which at least one atom is selected from the group, O, N or S.

Salts of compounds of formula (I) include pharmaceutically acceptable salts, for example acid addition salts derived from inorganic or organic acids, such as hydrochlorides, hydrobromides, p-toluenesulphonates, phosphates, sulphates, perchlorates, acetates, trifluoroacetates, propionates, citrates, malonates, succinates,

5   lactates, oxalates, tartrates and benzoates.

Salts may also be formed with bases. Such salts include salts derived from inorganic or organic bases, for example alkali metal salts such as magnesium or calcium salts, and organic amine salts such as morpholine, piperidine, dimethylamine or diethylamine salts.

10   When the "protected carboxy" group in compounds of the invention is an esterified carboxyl group, it may be a metabolically labile ester of formula CO_2R^{14} where R^{14} may be an ethyl, benzyl, phenethyl, phenylpropyl, α- or β-naphthyl, 2,4-dimethylphenyl, 4-*tert*-butylphenyl, 2,2,2-trifluoroethyl, 1-(benzyloxy)benzyl, 1-(benzyloxy)ethyl, 2-methyl-1-propionyloxypropyl, 2,4,6-trmethylbenzyloxymethyl

15   or pivaloyloxymethyl group.

Compounds of the general formula (I) may be prepared by any suitable method known in the art and/or by the following processes, which itself forms part of the invention.

According to a second aspect of the invention, there is provided a process for

20   preparing a compound of general formula (I) as defined above. It will be appreciated that where a particular stereoisomer of formula (I) is required, the synthetic processes described herein may be used with the appropriate homochiral starting material and/or isomers maybe resolved from mixtures using conventional separation techniques (eg. HPLC).

25   The compounds according to the invention may be prepared by the following process. In the description and formulae below the groups R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, A, B, C and X are as defined above, except where otherwise indicated. It will be appreciated that functional groups, such as amino, hydroxyl or carboxyl groups, present in the various compounds decribed

30   below, and which it is desired to retain, may need to be in protected form before any reaction is initiated. In such instances, removal of the protecting group may be the final step in a particular reaction. Suitable protecting groups for such functionality

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will be apparent to those skilled in the art.   For specific details see "Protective Groups in Organic Synthesis", Wiley Interscience, T W Greene, PGM Wuts.

Thus a process for preparing compounds of general formula (I) comprises the steps of:

5   deprotecting (for example by hydrolysis) a compound of general formula (II)

$$R^7S \underset{R^8}{\overset{O}{\longrightarrow}} \overset{R^1}{\underset{H}{N}} \overset{R^2}{\underset{O}{\overset{O}{\longrightarrow}}} \overset{O}{\underset{R^3}{N}} X \qquad (\,II\,)$$

10

wherein R^7 represents a suitable protecting group (eg *tert* butyl or acetate).

It will be appreciated that where a particular stereoisomer of formula (I) is required,
15   this may be obtained by conventional resolution techniques such as high performance liquid chromatography.   Where desired, however, appropriate homochiral starting materials may be used in the coupling reaction to yield a particular stereoisomer of formula (I).   This is exemplified below.

Intermediates of general formula (II) may be prepared by coupling an acid of
20   formula (III)

$$R^7S \underset{R^8}{\overset{O}{\longrightarrow}} OH \qquad (\,III\,)$$

25

wherein R^7 and R^8 are as defined above, or an active derivative thereof, with an amine of formula (IV)

$$H_2N \underset{O}{\overset{R^1}{\longrightarrow}} \overset{R^2}{\underset{R^3}{N}} X \qquad (\,IV\,)$$

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Active derivatives of acids of formula (III) include for example acid anhydrides or acid halides, such as acid chlorides.

The coupling reaction may be performed using standard conditions for amination reactions of this type. Thus, the reaction may be achieved in a solvent, for example

5   an inert organic solvent such as an ether, eg. a cyclic ether such as tetrahydrofuran, an amide eg. a substituted amide such as dimethylformamide, or a halogenated hydrocarbon such as dichloromethane at a low temperature eg. -30°C to ambient temperature, such as -20°C to 0°C, optionally in the presence of as base, eg. an organic base such as an amine, eg. triethylamine or a cyclic amine such as N-

10   methylmorpholine. Where an acid of formula (III) is used, the reaction may additionally be performed in the presence of a condensing agent, for example a diimide such as N,N'-dicyclohexylcarbodiimide, advantageously in the presence of a triazole such as 1-hydroxybenzotriazole. Alternatively, the acid may be reacted with a chloroformate for example ethylchloroformate, prior to reaction with the

15   amine of formula (IV).

The amine of general formula (IV) may be prepared by coupling an acid of formula (V), or an active derivative thereof

20

$$H_2N \overset{O}{\underset{R^1}{\diagup\!\!\!\diagdown}} OH \qquad (V)$$

with an amine of formula (VI)

25

$$\overset{R^2}{\underset{R^3}{HN}} \overset{O}{\diagup\!\!\!\diagdown} X \qquad (VI)$$

followed by removal of any protecting groups.

30   Active derivates of acids for formula (V) include for example acid anhydrides or acid halides such as acid chlorides as outlined earlier.

Amino acids and their derivatives as depicted by general formulae (V) and (VI) can be obtained in chiral or racemic form. In the chiral form they provide asymmetric building blocks for the chiral synthesis of compounds of general formula (1). Many of these derivatives can be readily obtained from commercially available starting

5   materials using methods known to those skilled in the art. (See "The Practice of Peptide Synthesis" by M. Bodanszk et al, Springer Verlag, New York, 1984, P. L. Durette, WO92/21360).

As a further extension to the present invention compounds of general formula (II) may be prepared by nucleophilic substitution of compounds of general formula (VII)

10

(VII)

15

wherein R^{15} represents a suitable leaving group (e.g. a halogen such as bromide, or an alkylsulphonate ester such as methanesulphonate) with a thiol of general formula (VIII)

20

$$R^7SH \qquad (VIII)$$

Wherein R^7 represents a suitable protecting group (eg. *tert*-butyl or acetate), using standard conditions known to those skilled in the art as exemplified in WO 90/05719.

25   Thiols of general formula (VIII) may be obtained from commercially available starting materials using methods known to those skilled in the art. Many thiols of general formula (VIII) are also commercially available.

Compounds of general formula (VII) may be prepared by coupling an acid of general formula (IX)

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$$R^{15} \diagdown \overset{O}{\underset{\underset{R^8}{|}}{\overset{*}{C}}} \diagup OH \qquad (IX)$$

wherein R^{15} and R^8 are as defined above (or suitably protected versions thereof) or an active derivative thereof, with an amine of formula (IV) using similar coupling conditions to those described for the preparation of compounds of formula (II).

Carboxylic acids of the structure depicted in formulae (III) and (IX) can be obtained in chiral or racemic form. Many of these derivatives can be readily obtained from commercially available starting materials using methods known to those skilled in the art (see WO 90/05719).

As a further extension to the present invention, intermediates of general formula (II) may be prepared by coupling an acid of formula (X)

$$(X) \qquad R^7S \diagdown \underset{R^8}{\overset{}{|}} \diagup \overset{O}{\underset{H}{\overset{}{C}}} N \diagup \underset{R^1}{\overset{}{|}} \diagup \overset{O}{\overset{}{C}} OH$$
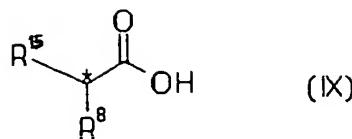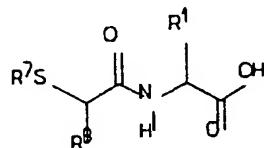
wherein R^1, R^7 and R^8 are as defined above, or an active derivative thereof, with an amine of formula (VI) by the procedure described previously.

Acids of general formula (X) may in turn be prepared by coupling an acid of formula (III), or an active derivative thereof with an amine of formula (VI), where X=OH or a suitably protected derivative therof followed by removal of any protecting groups.

Active derivates of acids for formula (V) include for example acid anhydrides or acid halides such as acid chlorides as outlined earlier.

Compounds of formula (I) may also be prepared by interconversion of other compounds of formula (I). Thus, for example, a compound of formula (I) wherein R^1 is a C_{1-6} alkyl group may be prepared by hydrogenation (using palladium on carbon in suitable solvent, such as an alcohol - eg ethanol) of a compound of

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formula (I) wherein R^1 is a C_{2-6} alkenyl group. A further example would include a compound of formula (I) wherein R^7 is a group R^{10} CO may be prepared by acylation (using a suitable acid chloride R^{10} COCl, in the presence of a base such as a triethylamine in a suitable solvent, such as a chlorinated solvent - eg

5   dichloromethane) of a compound of formula (I) wherein R^7 is H.

Any mixtures of final products or intermediates obtained can be separated on the basis of the pysico-chemical differences of the constituents, in known manner, into the pure final products or intermediates, for example by chromatography, distillation, fractional crystallization, or by formation of a salt if appropriate or possible under

10   the circumstances.

The compounds according to the invention exhibit *in vitro* inhibiting activities with respect to stromelysin, collagenase and gelatinase. Compounds according to the invention also exhibit *in vitro* inhibition of TNFα release. The activity and selectivity of the compounds may be determined by use of the appropriate enzyme

15   inhibition test, for example as described in Example A hereinafter.

This invention also relates to a method of treatment for patients (including man and/or mammalian animals raised in the dairy, meat or fur industries or as pets) suffering from disorders or diseases which can be attributed to matrix metalloproteinases and/or TNFα as previously described, and more specifically, a

20   · method of treatment involving the administration of the matrix metalloproteinase inhibitors of formula (I) as the active constituents.

Accordingly, the compounds of formula (I) can be used among other things in the treatment of osteoarthritis and rheumatoid arthritis, and in diseases and indications resulting from the over-expression of these matrix metalloproteinases such as found

25   in certain metastatic tumour cell lines.

As mentioned above, compounds of formula (I) are useful in human or veterinary medicine since they are active as inhibitors of TNFα and MMPs. Accordingly in another aspect, this invention concerns:

a method of management (by which is meant treatment or prophylaxis) of disease or

30   conditions mediated by TNFα and/or MMPs in mammals, in particular in humans, which method comprises administering to the mammal an effective amount of a compound of formula (I) above, or a pharmaceutically acceptable salt thereof; and

a compound of formula (I) for use in human or veterinary medicine, particularly in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by TNFα and/or MMPs; and

the use of a compound of formula (I) in the preparation of an agent for the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by TNFα and/or MMPs.

The disease or conditions referred to above include inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease; and those involving tissue breakdown such as bone resportion, inflammatory diseases, dermatological conditions, tumour growth, angiogenesis and invasion by secondary metastases, in particular rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration, tumour growth, angiogenesis and invasion by secondary metastases.

For the treatment of rheumatoid arthritis, osteoarthritis, and in diseases and indications resulting from the over-expression of matrix metalloendoproteinases such as found in certain metastatic tumour cell lines or other diseases mediated by the matrix metalloendoproteinases or increased TNFα production, the compunds of formula (I) may be administered orally, topically, parenterally, by inhalation spray or rectally in dosage unit formulations containing non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, cattle, sheep, dogs, cats etc, the compounds of the invention are effective in the treatment of humans.

The pharmaceutical composition containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving

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agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium

5   carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastointestinal tract

10 and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyeryl distearate may be employed. They may also be coated by the techniques described in the US Patents 4,256,108;4,166,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

15 Formulations for oral use may also be presented as hard gelatin capsules where in the active ingredient is mixed with an inert solid diluent, for example calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

20 Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents,   for   example   sodium   carboxymethylcellulose,   methylcellulose, hydroxypropylmethylcellulose,   sodium   alginate,   polyvinyl-pyrrolidone,   gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally occuring

25 phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene   oxide   with   long   chain   aliphatic   alcohols,   for   example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters dervied from fatty acids and a hexitol such a polyoxyethylene with partial

30 esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more

colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a
5   mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

10   Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified, for example sweetening, flavouring and colouring agents, may also be present.

15   The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally- occuring gums, for example gum acacia or gum tragacanth, naturally-occuring phosphatides, for example soya bean,
20   lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example gycerol,
25   propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been
30   mentioned above. The sterile injectable preparation may also be in a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles

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and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such

5   as oleic acid find use in the preparation of injectables.

The compounds of formula (I) may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the

10   rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc containing the compounds of Formula (I) are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

15   Dosage levels of the order of from about 0.05 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above- indicated conditions (about 2.5 mg to about 7 gms per patient per day). For example, inflammation may be effectively treated by the administration of from about 0.01 to 50 mg of the compound per kilogram of body weight per day (about 0.5 mg to about 3.5 gms per

20   patient per day).

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may vary from about 5 to about 95 percent of the total

25   composition. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet time of administration,

30   route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

The following Examples 1 to 79 illustrate the invention and their preparation (via the green Intermediates, as appropriate). Examples 1 to G illustrate test procedures.

In the Examples, the following abbreviations are used:

RT   Room temperature

5   DCC   Dicyclohexylcarbodiimide

EDC   1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride

TNF$_\alpha$ Tumour necrosis factor α

LPS   Lipopolysaccharide

ELISA Enzyme-linked immunosorbent assay

10

Intermediate 1   (RS)-2-Bromo-4-methoxycarbonylbutanoic acid

A solution of d-methyl-D,L-glutamic acid (6.0 g) (preparation according to Hanby *et al*, J. Chem. Soc. (1950), 51:3239) and potassium bromide (15.5 g) in aqueous sulfuric acid (1.25 M, 100 ml) was treated at 0°C portionwise with sodium nitrite

15   (4.0 g) over 1 h. The solution was allowed to warm to RT and was stirred over 2 h, then extracted with ethyl acetate (2 x 100 ml). The combined extracts were dried (MgSO$_4$) and evaporated *in vacuo* to give the title compound as a colourless oil (4.5 g).

TLC R$_f$ 0.14 (25% EtOAc-hexanes)

20   Similarly prepared were:

Intermediate 2   (RS)-2-Bromo-5-methoxycarbonylpentanoic acid

From (RS)-2-amino-5-methoxycarbonylpentanoic acid (1.8 g) (preparation based on the esterification procedure of Hanby *et al*, J. Chem. Soc. (1950), 51:3239) as a pale brown oil (1.95 g).

25   TLC R$_f$ 0.30 (5% MeOH-CH$_2$Cl$_2$)

Intermediate 3   (RS)-2-Bromo-6-methoxycarbonylhexanoic acid

From (RS)-2-amino-6-methoxycarbonylhexanoic acid (3.93 g) (preparation based on the esterification procedure of Hanby *et al*, J. Chem. Soc. (1950), 51:3239) as a colourless oil

30   (4.39 g).

TLC R$_f$ 0.26 (5% MeOH-CH$_2$Cl$_2$)

Intermediate 4   (RS)-2-Acetylmercapto-3-methoxycarbonylpropionic acid

23

A solution of potassium thiolacetate (1.48 g) in methanol (2 ml) was added to a solution of *mono*-methylmaleate (1.64 g) (prepared according the procedure exemplified in J. Am. Chem. Soc. (1986), 108:3) and the mixture was stirred overnight at RT. The solvent was removed by evaporation and the residue was partitioned between water (30 ml) and dichloromethane (30 ml), and the aqueous layer was then acidified to pH3 with 2N aqueous hydrochloric acid. The layers were separated and the aqueous layer was extracted with dichloromethane (2 x 30 ml). The combined organic extracts were dried (MgSO$_4$) and evaporated *in vacuo* to give a brown oil. Purification by flash column chromatography (eluting with 10% methanol in dichloromethane) gave the title compound as a colourless oil (0.48 g).

TLC R$_f$ 0.30 (10% MeOH-CH$_2$Cl$_2$)

__Intermediate 5__   (*RS*)-2-Acetylmercapto-4-methoxycarbonylbutanoic acid

Potassium thiolacetate (2.0 g) was added to a solution of intermediate 1 (3.0 g) in ethanol (30 ml) and the mixture was stirred at RT overnight. The solution was evaporated *in vacuo* and the residue was partitioned between ethyl acetate (30 ml) and water (30 ml). The organic layer was then washed with saturated brine (30 ml), dried (MgSO$_4$) and evaporated *in vacuo* to give the title compound as a colourless oil (1.83 g).

TLC R$_f$ 0.46 (EtOAc)

Similarly prepared were:

__Intermediate 6__   (*RS*)-2-Acetylmercapto-5-methoxycarbonylpentanoic acid

From Intermediate 2 (1.89 g) as a yellow oil (0.87 g).

TLC R$_f$ 0.30 (5% MeOH-CH$_2$Cl$_2$)

__Intermediate 7__   (*RS*)-2-Acetylmercapto-6-methoxycarbonylhexanoic acid

From Intermediate 3 (4.3 g) as a yellow oil (2.78 g).

TLC R$_f$ 0.23 (5% MeOH-CH$_2$Cl$_2$)

__Intermediate 8__   (*RS*)-(1,1-Dimethylethyl) 2,4-dibromobutyrate

Bromine (31.3ml, 0.54mol) was added dropwise over 4h at 100°C to neat stirring 4-bromobutyryl chloride (100g, 0.54mol). The resulting acid chloride was cooled then added dropwise at 0°Cto a stirred solution of *tert*-butanol (240ml) and triethylamine (64ml, 0,461mol) in anhydrous dichloromethane (600ml). 2M Hydrochloric acid was added and the layers separated. The organic portion was then washed

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sequentially with 10% sodium metabisulphite solution (2x500ml), water (500ml) and brine (500ml), dried (MgSO₄) and evaporated *in vacuo* to provide the title compound (120g, 86%) as a brown liquid.

¹H NMR (250MHz; CDCl₃), Ref., TMS) 1.50 (9H, s), 2.45 (2H, q), 3.55 (2H, t) and 4.40 (1H, dd).

Similarly prepared were:

Intermediate 9   (*RS*)-(1,1-Dimethylethyl) 2,5-dibromopentanoate

From 5-bromovaleryl chloride (75g, 0.37mol), as a brown oil (81g, 68%).

¹H NMR (250MHz; CDCl₃), Ref., TMS) 1.50 (9H, s), 1.8-2.3 (4H,m), 3.45 (2H, t) and 4.18 (1H, dd).

Intermediate 10   (*RS*)-(1,1-Dimethylethyl) 2,6-dibromohexanoate

From 6-bromohexanoyl chloride (100g, 0.47mol), as a brown oil (133g, 87%).

¹H NMR (250MHz; CDCl₃, Ref., TMS) 1.50 (9H, s), 1.6-2.1 (6H, m), 3.4 (2H, t) and 4.1 (1H, dd).

Intermediate 11   (*RS*)-(1,1-Dimethylethyl) 2,4-bis-(acetylmercapto)butyrate

Potassium thiolacetate (1.51g, 13.2mmol)was added to a stirred solution of intermediate 8 (2g, 6.6mmol) in methanol (25ml) and the mixture stirred at RT overnight. The mixture was diluted with dichloromethane (100ml), washed with brine (2x50ml), dried (MgSO₄) and evaporated *in vacuo* to a yellow oil. Purification by flash column chromatography (eluting with 30% dichloromethane in hexane) provided the title compound (1.1g, 57%) as a colourless oil.

TLC R$_f$ 0.57 (CH₂Cl₂)

Similarly prepared were:

Intermediate 12   (*RS*)-(1,1-Dimethylethyl) 2,5-bis-(acetylmercapto)pentanoate

From intermediate 9 (2g, 6.32mmol), as a colourless oil (1.12g, 57%)

TLC R$_f$ 0.57 (CH₂Cl₂)

Intermediate 13   (*RS*)-(1,1-Dimethylethyl) 2,6-bis-(acetylmercapto)hexanoate

From intermediate 10 (2g, 6.32mmol), as a colourless oil (1.09g, 57%)

TLC R$_f$ 0.57 (CH₂Cl₂)

Intermediate 14   (*RS*)-2,3-Bis-(acetylmercapto)propionic acid

A solution of thiolacetic acid (1.12 g) in 1N aqueous potassium hydroxide (14.7 ml) was added dropwise to a solution of 2,3-dibromopropionic acid (1.71 g) in 1N

aqueous potassium hydroxide (7.35 ml) and the mixture was stirred 5 h at RT. The pH of the mixture was adjusted to 8-9 by the addition of further 1N aqueous potassium hydroxide and the mixture was stirred a further 2 h, then acidified to pH1-2 by the addition of concentrated hydrochloric acid and extracted with ethyl acetate
(2 x 25 ml). The combined extracts were dried (Na_2SO_4) and evaporated to give a yellow oil. The product from two reactions was combined and purification by flash column chromatography (eluting with 4% acetic acid-toluene) gave the title compound as a colourless oil (0.422 g).

TLC R_f 0.15 (5% AcOH-toluene)

Intermediate 15   (RS)-2,4-Bis-(acetylmercapto)butyric acid

A solution of intermediate 11 (1.1g, 3.7mmol) in dichloromethane (50ml) was treated with trifluoroacetic acid (2.9ml, 37mmol) and the mixture stirred at RT overnight. Water (50ml) was added and the mixture extracted with dichloromethane (3x40ml). The combined organic extracts were then washed with water (50ml) and brine (50ml), dried ($MgSO_4$) and evaporated in vacuo to provide the product (870mg, 98%) as a pale yellow oil.

TLC R_f 0.12 (25% MeOH-CH_2Cl_2)

Similarly prepared were:

Intermediate 16   (RS)-2,5-Bis-(acetylmercapto)pentanoic acid

From intermediate 12 (1.1g, 3.6mmol), as a pale yellow oil (906mg, 100%)

TLC R_f 0.12 (25% MeOH-CH_2Cl_2)

Intermediate 17   (RS)-2,6-Bis-(acetylmercapto)hexanoic acid

From intermediate 13 (1.1g, 3.6mmol), as a pale yellow oil (895mg, 98%)

TLC R_f 0.12 (25% MeOH-CH_2Cl_2)

Intermediate 18   4-Phthalimidobutanoic acid

N-Carboethoxyphthalimide (10.96 g) was added in one portion to a vigorously stirred solution of 4-aminobutanoic acid (5.16 g) and sodium carbonate (5.35 g) in water (150 ml) at RT. The mixture was stirred until essentially all the solid material had dissolved (30 min), then it was filtered. The filtrate was acidified to pH1 with 6N aqueous hydrochloric acid (ca. 22 ml) and the white precipitate·was collected by

filtration and washed thoroughly with water (150 ml). The solid was dried in air, then *in vacuo* to give the title compound as a colourless solid (7.35 g).

^{1}H NMR (250 MHz; CDCl$_3$, Ref.,TMS) d 2.03 (2H, pent.) 2.42 (2H, t), 3.78 (2H, t), 7.65-7.77 (2H, m), 7.81-7.90 (2H, m)

Similarly prepared were:

Intermediate 19   5-Phthalimidopentanoic acid

From 5-aminopentanoic acid (5.0 g) as a colourless solid (6.8 g).

^{1}H NMR (250 MHz; CDCl$_3$, Ref., TMS) d 1.6-1.8 (4H, m), 2.20 (2H, t), 3.85 (2H, t), 7.70-7.75 (2H, m), 7.85-7.95 (2H, m), 10.2 (1H, br s)

Intermediate 20   6-Phthalimidohexanoic acid

From 6-aminohexanoic acid (5.0 g) as a colourless solid (5.8 g).

^{1}H NMR (60 MHz; CDCl$_3$, Ref., TMS) 1.5-2.4 (6H, m) 2.3 (2H, t) 3.80 (2H, t), 7.8-8.1 (4H, m), 10.4 (1H, br s)

Intermediate 21   2-(3-Phthalimidophenyl)acetic acid

From 2-(3-aminophenyl)acetic acid (3.0g) as an off-white solid (4.0g, 72%).

TLC R$_f$ 0.36 (7.5% MeOH-0.5% AcOH-CH$_2$Cl$_2$)

Intermediate 22   *Cis*-3-Aminocyclopent-4,5-enecarboxylic acid

A solution of racemic lactam (50g, 0.458mol) in 2N hydrochloric acid (1000ml) was heated under reflux for 1h. The mixture was evaporated *in vacuo* and the residue crystallised from acetone to provide the hydrochloride salt of the title compound (73g, 97%) as a white solid.

This hydrochloride salt (20g, 0.122mol)was dissolved in water (300ml) and the stirred solution treated with amberlite (IRA-67) ion exchange resin until pH 7 was reached. The resin was then removed by filtration, the solvent removed *in vacuo* and the residue crystallised from acetone to provide the title compound (13.7g, 88%) as a white solid.

Intermediate 23   *Cis*-3-Phthalimidocyclopent-4,5-enecarboxylic acid

From intermediate 22 hydrochloride (22.3g, 0.136mol), as a white solid (13.7g, 39%).

TLC R$_f$ 0.37 (1% AcOH-5% MeOH-CH$_2$Cl$_2$)

Intermediate 24   *Trans*-Methyl 3-Phthalimidocyclopent-4,5-enecarboxylate

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Intermediate 23 (23.2g, 0.183mol) and phthalic anhydride (27.03g, 0.183mol) were powdered together and melted at 190°C under nitrogen with stirring. The mixture was allowed to cool to RT and the residue treated with ethyl acetate (120ml). Activated charcoal (1.0g) was added and the mixture heated under reflux for 30min,

5   filtered through celite and the filtrate evaporated *in vacuo* to provide the intermediate phthalimido-acid, a 1:1 mixture of *cis/trans* isomers, as a pale yellow solid (45.7g, 97%).

A solution of this acid in methanol (300ml) was treated with conc. hydrochloric acid (0.5ml) and the mixture heated under reflux for 30min, allowed to cool to RT and

10   the solvent evaporated *in vacuo*. The residue was dissolved in ethyl acetate (400ml) and the solution washed with 8% sodium bicarbonate (2x100ml), water (100ml) and brine (100ml), dried (MgSO₄) and evaporated *in vacuo* to provide the phthalimido-ester as a 1:1 mixture of *cis/trans* isomers. Separation by flash column chromatography (eluting with 60% ether-pentane) provided the title compound

15   (9.72g, 20%) as a white solid.

TLC R$_f$ 0.46 (40% pentane-ether)

Intermediate 25   *Trans*-3-Phthalimidocyclopent-4,5-enecarboxylic acid
A solution of intermediate 24 (9.72g, 35.8mmol) in a mixture of 0.5N hydrochloric acid (100ml) and glacial acetic acid (100ml) was heated under reflux for 30min. The

20   mixture was diluted with water (200ml) and extracted with ethyl acetate (3x100ml). The combined extracts were washed with brine (100ml), dried (MgSO₄) and evaporated *in vacuo* to provide the crude product which was crystallised from ether (7.56g, 82%) as a white solid.

TLC R$_f$ 0.48 (1% AcOH-5% MeOH-CH₂Cl₂)

25   Intermediate 26   *Cis*-3-Phthalimidocyclopentanecarboxylic acid
Intermediate 23 (15.1g, 58.7mmol) was hydrogenated at RT and atmospheric pressure over 5% palladium on charcoal (2g) in ethyl acetate (700ml) overnight. The catalyst was removed by filtration through celite and the filtrate evaporated *in vacuo* to provide the title compound (15g, 98%) as white solid.

30   TLC R$_f$ 0.37 (1% AcOH-5% MeOH-CH₂Cl₂)

Similarly prepared was:

Intermediate 27   *Trans*-3-Phthalimidocyclopentanecarboxylic acid

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From intermediate 25 (7.55g, 29.3mmol), as a white solid (7.04g, 93%).

TLC R$_f$ 0.47 (1% AcOH-5% MeOH-CH$_2$Cl$_2$)

Intermediate 28   Cis-(1,1-Dimethylethyl) 3-Phthalimidocyclopentylacetate

A solution of intermediate 26 (5.09g, 19.6mmol) in dry dichloromethane (60ml) was
treated with oxalyl chloride (3.4ml, 39.3mmol) then dimethylformamide (1 drop).
The mixture was stirred at RT for 2h then the solvent evaporated in vacuo to provide
the intermediate acid chloride.

The residue was dissolved in tetrahydrofuran (30ml) then treated with a solution of
diazomethane in ether (200ml, ca. 80mmol) at 0°C. The mixture was stirred at RT
overnight then evaporated in vacuo to provide the diazoketone as a yellow solid.

The diazoketone was dissolved in tert-butanol (100ml) and heated under reflux while
a solution of silver benzoate (438mg, 1.9mmol) in triethylamine (5ml) was added in
small portions over 2h. The mixture was heated under reflux for a further 1h then
cooled to RT, filtered through celite and the filtrate evaporated in vacuo to provide
a yellow solid. The residue was dissolved in dichloromethane (75ml) and the
solution washed sequentially with 8% sodium bicarbonate (50ml), water (50ml) and
brine, dried (MgSO$_4$) and evaporated in vacuo to provide the crude product.
Purification by flash column chromatography (eluting with 50% ether-pentane)
provided the title compound (3.8g, 60%) as a white solid.

TLC R$_f$ 0.52 (50% pentane-ether)

Similarly prepared was:

Intermediate 29   Trans-(1,1-Dimethylethyl) 3-Phthalimidocyclopentylacetate

From intermediate 27 (1.41g, 5.44mmol), as a white solid (1.22g, 70%).

TLC R$_f$ 0.58 (50% pentane-ether)

Intermediate 30   Cis-3-Phthalimidocyclopentylacetic acid

A solution of intermediate 28 (1.78g, 5.4mmol) in dichloromethane (20ml) was
treated with trifluoroacetic acid (2.1ml, 27mmol) and the mixture stirred at RT
overnight. The solvent and excess trifluoroacetic acid was removed in vacuo to
provide the title compound (1.41g, 96%) as a white solid.

TLC R$_f$ 0.32 (30% pentane-ether)

Similarly prepared was:

Intermediate 31   *Trans*-3-Phthalimidocyclopentylacetic acid

From intermediate 29 (1.18g, 3.58mmol), as a white solid (842mg, 86%).

TLC R_f 0.41 (30% pentane-ether)

Intermediate 32   (*RS*) 2-Bromo-5-phthalimidopentanoic acid

Intermediate 19 (5.0 g 20.2 mmol) and thionyl chloride (10 ml) were heated together at 65°C for 30 min. *N*-Bromosuccinimide (5.4 g) and further thionyl chloride were added, plus 48% aqueous HBr (1 drop). The solution was heated at 60°C for 10 min then 70°C for 2 h 15 min. Further *N*-bromosuccinimide (850 mg) was added and the mixture was heated at 70°C for 2 h. Excess thionyl chloride was removed by evaporation under reduced pressure and the oily residue was diluted with dry tetrahydrofuran (200 ml) and water (200ml). The mixture was then treated cautiously with solid sodium bicarbonate to pH 7-8 then stirred overnight at RT. Excess tetrahydrofuran was removed *in vacuo* and the residue washed with dichloromethane (3x300ml). The aqueous portion was then cautiously acidified to pH 1 using 6M hydrochloric acid and extracted with dichloromethane (4x200ml). The combined extracts were then washed with water (2x400ml) and brine (400ml), dried (MgSO₄) and evaporated *in vacuo* to provide the product (4.7g, 71%) as a fawn solid.

TLC R_f 0.47 (EtOAc)

Similarly prepared were:

Intermediate 33   (*RS*) 2-Bromo-2-(3-phthalimidophenyl)acetic acid

From intermediate 21 as a colourless solid (588mg, 75%)

TLC R_f 0.19 (5% MeOH-0.1% AcOH-CH₂Cl₂)

Intermediate 34   *Cis*-a-Bromo-3-phthalimidocyclopentylacetic acid

From intermediate 30 (1.41g, 5.16mmol), as a buff foam (1.59g, 87%).

TLC R_f 0.46 (2% MeOH-ether)

Intermediate 35   *Trans*-a-Bromo-3-phthalimidocyclopentylacetic acid

From intermediate 31 (815mg, 2.98mmol), as a yellow-brown foam (805mg, 77%).

TLC R_f 0.48 (2% MeOH-ether)

Intermediate 36   (*RS*) 2-Acetylmercapto-5-phthalimidopentanoic acid

A solution of intermediate 33 (3.0g, 9.2mmol) in methanol (30ml) was treated with potassium thiolacetate (1.05g, 9.2mmol) and the mixture stirred at RT overnight. The mixture was evaporated *in vacuo*, the residue dissolved in dichloromethane

(100ml) then the solution washed with water (2x50ml), dried (MgSO$_4$) and evaporated *in vacuo* to provide the product (2.4g, 81%) as a pale yellow foam.

TLC R$_f$ 0.43 (EtOAc)

Similarly prepared were:

Intermediate 37   (*RS*) 2-Acetylmercapto-2-(3-phthalimido)phenylacetic acid

From intermediate 33 as a colourless solid (722mg, 100%)

TLC R$_f$ 0.15 (5% MeOH-0.1% AcOH-CH$_2$Cl$_2$)

Intermediate 38   *Cis*-a-(Acetylmercapto)-3-phthalimidocyclopentylacetic acid

From intermediate 34 (2.01g, 5.71mmol), as a brown foam (1.44g, 73%).

TLC R$_f$ 0.42 (Ether)

Intermediate 39   *Trans*-a-(Acetylmercapto)-3-phthalimidocyclopentylaceticacid

From intermediate 35 (774mg, 2.2mmol), as a beige foam (329mg, 43%).

TLC R$_f$ 0.45 (Ether)

Intermediate 40   (*RS*)-*N*-[2-Bromo-4-phthalimidobutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

Intermediate 18 (2.33 g) and thionyl chloride (2.92 ml) were heated together at 65°C for 30 min. *N*-Bromosuccinimide (2.51 g) and further thionyl chloride were added, plus 48% aqueous HBr (1 drop). The solution was heated at 60°C for 10 min then 70°C for 2 h 15 min. Further *N*-bromosuccinimide (850 mg) was added and the mixture was heated at 70°C for 2 h. Excess thionyl chloride was removed by evaporation under reduced pressure and the oily residue was diluted with dry dichloromethane (10 ml). A portion of the supernatant (4.0 ml) was added to a solution of *L*-leucyl-*L*-phenylalanine *N*-methyl amide (500 mg) and triethylamine (0.24 ml) in dry dichloromethane (10 ml) at 0°C, and this mixture was stirred overnight at RT. The mixture was diluted with dichloromethane and washed with saturated aqueous sodium bicarbonate solution (50 ml), 1N aqueous hydrochloric acid (50 ml), and saturated brine (50 ml), then dried (MgSO$_4$) and evaporated *in vacuo* to give a brown solid. This material was purified by flash column chromatography (4 x 18 cm; eluting with 2% methanol-dichloromethane) to give the title compound as an off-white solid (530 mg).

TLC R$_f$ 0.44 (5% MeOH-CH$_2$Cl$_2$)

Similarly prepared were:

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Intermediate 41   (RS)-N-[2-Bromo-5-phthalimidopentanoyl]-ʟ-1 ucyl-ʟ-
                       phenylalanine N-methyl amide

From Intermediate 19 (4.0 g) and ʟ-leucyl-ʟ-phenylalanine N-methyl amide (1.18 g),
as a pink solid (1.2 g).

5   TLC R$_f$0.34 (5% MeOH-CH$_2$Cl$_2$)

Intermediate 42   (RS)-N-[2-Bromo-6-phthalimidohexanoyl]-ʟ-leucyl-ʟ-
                       phenylalanine N-methyl amide

From Intermediate 20 (4.0 g) and ʟ-leucyl-ʟ-phenylalanine N-methyl amide (1.18 g),
as a near colourless solid (1.0 g).

10   TLC R$_f$ 0.52 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 43   (RS)-2-[(1,1-Dimethylethyl)mercapto]-5-phthalimidopentanoic
                       acid

Tert-butylthiol (11.3ml, 0.1mol) was added to a stirred solution of potassium tert-
butoxide (22.45g, 0.1mol) in anhydrous tetrahydrofuran (215ml) and the mixture

15   stirred at RT for 20 min. A solution of intermediate 13 (32.6g, 0.1mol) in
anhydrous tetrahydrofuran (80ml) was then added and the mixture stirred at RT
overnight. Water (300ml) was added, the mixture acidified to pH 1 with 1N
hydrochloric acid and extracted with dichloromethane (3x200ml). The combined
extracts were then dried (MgSO$_4$) and evaporated in vacuo to provide a yellow oil.

20   Purification by flash column chromatography (eluting with 5-15% dichloromethane
in ethyl acetate) then crystallisation from dichloromethane/hexane furnished the title
compound
(22g, 66%) as a pale yellow solid.
TLC R$_f$ 0.48 (10% MeOH-CH$_2$Cl$_2$)

25   Intermediate 44   (RS)-N-[2-[(1,1-Dimethylethyl)mercapto]-5-
                       phthalimidopentanoyl]-L-leucyl-L-phenylalanine   N-methyl
                       amide

A solution of intermediate 43 (8.06g, 24mmol) and L-leucyl-L-phenylalanine N-
methyl amide (7.0g, 24mmol) in dichloromethane (250ml) was treated with N-

30   hydroxybenzotriazole (3.9g, 28.9mmol) then EDC (5.06g, 26.4mmol) and the
mixture stirred at RT overnight. The mixture was washed with 1N hydrochloric acid
(300ml) and the aqueous portion re-extracted with dichloromethane (2x100ml). The

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combined extracts were washed sequentially with 1N hydrochloric acid (300ml), 8% sodium bicarbonate (300ml), water (300ml) and brine (300ml), dried (MgSO₄) and evaporated *in vacuo* to provide a pale yellow solid. Purification by flash column chromatography (eluting with 5% methanol in dichloromethane) provided the title

5　compound (12.8g, 88%) as a near white solid.

TLC R$_f$ 0.55 (10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 45</u>　　(*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

A solution of intermediate 44 (2.56g, 4.2mmol) in a mixture of tetrahydrofuran

10　(10ml) and ethanol (50ml) was treated with hydrazine hydrate (10ml, xs) and the mixture heated under reflux for 2h. After cooling to RT water (30ml) was added, the solvent removed *in vacuo*, the residue acidified to pH 1 with 1N hydrochloric acid and washed with dichloromethane (2x100ml). The aqueous layer was then basified to pH 14 with 2M sodium hydroxide and extracted with dichloromethane

15　(2x100ml). The combined extracts were washed with brine (100ml), dried (MgSO₄) and evaporated *in vacuo* to provide a pale yellow solid. Purification by flash column chromatography (eluting with 10-25% methanol in dichloromethane) provided the title compound (9.2g, 91%) as a near white solid.

TLC R$_f$ 0.23 (30% MeOH-CH$_2$Cl$_2$)

20　<u>Intermediate 46</u>　　(*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(acetylamino)pentanoyl]-*L*-leucyl-*L*-phenylalanine　*N*-methyl amide

Acetyl chloride (0.5ml, 7mmol) was added to a stirred solution of intermediate 45 (1.14g, 2.38mmol) and triethylamine (2ml, 14.4mmol) in anhydrous dichloromethane

25　(45ml), the mixture was then stirred at RT overnight. The mixture was diluted with dichloromethane (75ml) and washed successively with 1N hydrochloric acid (100ml), 8% sodium bicarbonate (100ml), water (100ml) and brine (100ml), dried (MgSO₄) and evaporated *in vacuo* to provide a pale yellow solid. Purification by flash column chromatography (eluting with 5% methanol in dichloromethane) provided the title

30　compound (1.2g, 95%) as a near white solid.

TLC R$_f$ 0.31 (10% MeOH-CH$_2$Cl$_2$)

Similarly prepared were:

Intermediate 47   (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(benzoylamino)pentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (2.86g, 5.97mmol) and benzoyl chloride (0.84ml, 7.2mmol), as a near white solid (3.43g, 99%).

TLC R_f 0.41 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 48   (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-succinimidopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (1.14g, 2.38mmol) and succinic anhydride (0.33g, 3.3mmol), as a near white solid (0.54g, 40%).

TLC R_f 0.58 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 49   (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(methanesulphonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (2.03g, 4.24mmol) and methanesulphonyl chloride (0.35ml,4.6mmol), as a near white solid (1.78g, 77%).

TLC R_f 0.46 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 50   (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(benzenesulphonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (2.03g, 4.24mmol) and benzenesulphonyl chloride (0.59ml, 4.6mmol), as a near white solid (2.18g, 85%).

TLC R_f 0.54 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 51   (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(methoxycarbonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (200mg, 0.43mmol) and methyl chloroformate (0.03ml, 0.41mmol), as a near white solid (196mg, 89%).

TLC R_f 0.32 (5% MeOH-CH$_2$Cl$_2$)

Intermediate 52   (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(benzyloxycarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (200mg, 0.43mmol) and benzyl chloroformate (0.06ml, 0.41mmol), as a near white solid (230mg, 93%).

TLC R$_f$ 0.44 (5% MeOH-CH$_2$Cl$_2$)

Intermediate 53   (RS)-N-[2-[(1,1-Dimethylethyl)mercapto]-5-(4-pyridylcarbonyl)
aminopentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 45 (2.03g, 4.24mmol) and isonicotinoyl chloride (834mg, 4.66mmol), as a near white solid (1.81g, 77%).

TLC R$_f$ 0.22 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 54   (RS)-N-[2-[(1,1-Dimethylethyl)mercapto]-5-(3-pyridylcarbonyl)
aminopentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 45 (200mg, 0.42mmol) and nicotinoyl chloride (83mg, 0.47mmol), as a near white solid (130mg, 56%).

TLC R$_f$ 0.26 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 55   (RS)-N-[2-[(1,1-Dimethylethyl)mercapto]-5-(2-pyridylcarbonyl)
aminopentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 45 (200mg, 0.42mmol) and picolinoyl chloride (83mg, 0.47mmol), as a near white solid (145mg, 62%).

TLC R$_f$ 0.21 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 56   (RS)-N-[2-[(1,1-Dimethylethyl)mercapto]-5-(2-
pyrazinylcarbonyl)
aminopentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 45 (200mg, 0.42mmol) and pyrazinoyl chloride (85mg, 0.47mmol), as a near white solid (145mg, 62%).

TLC R$_f$ 0.18 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 57   (RS)-N-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(acetyl)amino
pentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

2-Nitrosulphenyl chloride (3.26g, 6.26mmol) was added to a stirred solution of intermediate 46 (1.23g, 6.49mmol) in glacial acetic acid (75ml) and the mixture stirred at RT overnight. The solvent was removed in vacuo and the residue purified by flash column chromatography (eluting with 5% methanol in dichloromethane) to provide the title compound (3.74g, 97%) as a yellow solid.

TLC R$_f$ 0.34 (10% MeOH-CH$_2$Cl$_2$)

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Similarly prepared were:

Intermediate 58   (RS)-N-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-
(benzoylamino)pentanoyl]-L-leucyl-L-phenylalanine N-methyl
amide

5   From intermediate 47 (1.08g, 5.67mmol), as a yellow solid (3.43g, 99%).

TLC R_f 0.54 (10% MeOH-CH_2Cl_2)

Intermediate 59   (RS)-N-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-succinimido
pentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 48 (618mg, 1.1mmol), as a yellow solid (665mg, 92%).

10   TLC R_f 0.25 (10% MeOH-CH_2Cl_2)

Intermediate 60   (RS)-N-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-
(methanesulphonyl)aminopentanoyl]-L-leucyl-L-phenylalanine
N-methyl amide

From intermediate 49 (1.78g, 3.27mmol), as a yellow solid (1.84g, 88%).

15   TLC R_f 0.32 (10% MeOH-CH_2Cl_2)

Intermediate 61   (RS)-N-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-
(benzenesulphonyl)aminopentanoyl]-L-leucyl-L-phenylalanine
N-methyl amide

20   From intermediate 50 (2.25g, 3.71mmol), as a yellow solid (1.6g, 68%).

TLC R_f 0.37 (10% MeOH-CH_2Cl_2)

Intermediate 62   (RS)-N-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-
(methoxycarbonyl)aminopentanoyl]-L-leucyl-L-phenylalanine
N-methyl amide

25   From intermediate 51 (196mg, 0.31mmol), as a yellow solid (86mg, 38%).

TLC R_f 0.25 (10% MeOH-CH_2Cl_2)

Intermediate 63   (RS)-N-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-
(benzyloxycarbonyl)aminopentanoyl]-L-leucyl-L-phenylalanine
N-methyl amide

30   From intermediate 52 (229mg, 0.32mmol), as a yellow solid (155mg, 60%).

TLC R_f 0.37 (10% MeOH-CH_2Cl_2)

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<u>Intermediate 64</u>   (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(4-pyridylcarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 53 (1.92g, 3.29mmol), as a yellow solid (1.66g, 74%).

5   TLC R$_f$ 0.29 (10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 65</u>   (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(3-pyridylcarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 54 (190mg, 0.33mmol), as a yellow solid (85mg, 38%).

10   TLC R$_f$ 0.24 (10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 66</u>   (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(2-pyridylcarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 55 (210mg, 0.36mmol), as a yellow solid (120mg, 54%).

15   TLC R$_f$ 0.21 (10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 67</u>   (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(2-pyrazinylcarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 56 (300mg, 0.51mmol), as a yellow solid (110mg, 42%).

20   TLC R$_f$ 0.18 (10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 68</u>   (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

TLC R$_f$ 0.16 (10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 69</u>   (*R*)-*N*-(Phenylmethoxy)carbonyl-(*S*-methyl)-*L*-cysteine

25   Benzyl chloroformate (11.1ml, 65mmol) was added dropwise to a stirred solution of (*S*-methyl)-*L*-cysteine (10g, 75mmol) in 2M aqueous sodium hydroxide (50ml) and the mixture stirred at RT for 4h. The mixture was then basified to pH14 with further 2M sodium hydroxide and the solution washed with ethyl acetate (4x50ml). The aqueous phase was acidified to pH3 with concentrated hydrochloric acid and

30   then extracted with ethyl acetate (4x70ml). The combined extracts were washed with brine, dried (MgSO$_4$) and evaporated *in vacuo* to provide the product as a pale yellow oil (16.8g, 84%).

TLC R$_f$ 0.21 (10% MeOH-CHCl$_3$)

<u>Intermediate 70</u>   (*R*)-*N*-(1,1-Dimethylethoxy)carbonyl-(*S*-methyl)cysteine

Di-tert-butyldicarbonate (8.88g, 40.7mmol) was added to a stirred solution of *S*-methyl-*L*-cysteine (5g, 37mmol) and sodium bicarbonate (7.8g, 92.5mmol) in a

mixture of water (150ml) and dioxane (100ml) at 0°C. The mixture was allowed to

warm to RT and stirred overnight, then diluted with water (100ml), acidified to pH

3 using 1N hydrochloric acid then extracted with ethyl acetate (3x100ml). The

combined extracts were washed with brine, dried (MgSO$_4$) and evaporated *in vacuo*

to provide the title compound as a colourless oil (6.64g, 76%).

TLC R$_f$ 0.55 (MeOH-H$_2$O)

Similarly prepared was:

<u>Intermediate 71</u>   (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl-propylglycine

From (*S*)-norvaline (5g, 42.7mmol), as a colourless oil (6.3g, 68%).

TLC R$_f$ 0.50 (MeOH-H$_2$O)

<u>Intermediate 72</u>   (S)-*N*-(1,1-Dimethylethoxy)carbonyl-(*O*-methyl)serine

Sodium hydride (60% dispersion, 4.3g, 0.107mol) was added portionwise at 0°Cto

a stirred solution of (S)-*N*-(1,1-dimethylethoxy)carbonylserine (10g, 48.7mmol) in

anhydrous DMF (250ml). A solution of iodomethane (6.1ml, 0.1mol) in anhydrous

DMF (10ml) was then added dropwise. The mixture was allowed to warm to RT and

stirred overnight, then diluted with 1N hydrochloric acid to pH3 and the solution

concentrated *in vacuo* to *ca.* 200ml. The mixture was diluted with water (200ml) and

extracted with ethyl acetate (3x100ml). The combined extracts were washed with

brine (100ml), dried (MgSO$_4$) and evaporated *in vacuo* to provide the title compound

as a yellow oil (7.48g, 70%).

TLC R$_f$ 0.39 (10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 73</u>   (*RS*)-*N*-Benzoyl-[b-(4-pyridyl)]alanine methyl ester

a)   2-(4-Pyridyl)-1-(*N*-benzoyl)aminoethylene-1-carboxylic acid hydrochloride

4-pyridylcarboxaldehyde (85g, 0.79mol) was added to a stirred ice cold suspension

of sodium acetate (16g,0.195mol) and hippuric acid (150g, 0.84mol) in acetic

anhydride (360ml, 3.8mol) an exothermic reaction ensued and the internal

temperature reached 30-40°C. The mixture was cooled to RT then poured into water

(1500ml). The resulting solid was removed by filtration washed with water (2x500ml) to provide the intermediate azlactone (74g, 37%) as a brown solid.

The azlactone (70g, 0.28mol) was dissolved in conc. hydrochloric acid (210ml) resulting in the rapid formation of a precipitate which was removed by filtration,

5   washed with acetone (2x500ml) and dried *in vacuo* to provide the product (71g, 78%) as a pale green solid.

b)   (*RS*)-*N*-Benzoyl-[b-(4-pyridyl)]alanine hydrochloride

The olefin hydrochloride (70g, 0.23mol) was hydrogenated at RT and atmospheric pressure over 5% palladium on carbon (7g) in water (700ml) overnight. The catalyst

10   was removed by filtration through celite and the solvent concentrated to a volume of *ca*. 100ml. Acetone (1000ml) was added and the resulting precipitate removed by filtration and dried *in vacuo* to provide the product (53g, 75%) as an off-white solid.

c)   (*RS*)-*N*-Benzoyl-[b-(4-pyridyl)]alanine methyl ester

15   Thionyl chloride (15ml, 0.21mol) was added to an ice cold solution of the acid (53g, 0.17mol) in methanol (200ml). The mixture was allowed to warm to RT over 30min before the solvent was removed *in vacuo* providing an off-white semi-solid. The residue was suspended in 8% sodium bicarbonate (500ml) and the product extracted into ethyl acetate (4x400ml). The combined organic extracts were then

20   washed with brine (1000ml), dried (MgSO$_4$) and evaporated *in vacuo* providing the title compound (46g, 94%) as an off-white solid.

$C_{16}H_{16}N_2O_3$ [284.3]; [MH$^+$ 285]
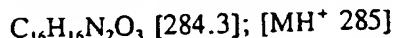
Similarly prepared were:

Intermediate 74   (*RS*)-*N*-Benzoyl-[b-(3-pyridyl)]alanine methyl ester

25   In three steps from pyridine-3-carboxaldehyde as a white solid.

$C_{16}H_{16}N_2O_3$ [284.3]; [MH$^+$ 285]
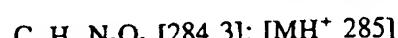
Intermediate 75   (*RS*)-*N*-Benzoyl-2-methoxyalanine methyl ester

In three steps from 2-methoxybenzaldehyde as a white solid

$C_{17}H_{19}N_2O_4$ [301.3]; [MH$^+$302]

30   Intermediate 76   (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl-[b-(4-pyridyl)]alanine

Intermediate 73 (27g, 0.104mol) was dissolved in hot acetone (50ml) and the solution added to 0.03M potassium dihydrogen phosphate (500ml) at pH7.2.

Alcalase (1ml) was then added to the vigorously stirred solution and the pH maintained at 7.2 by the addition of 1M sodium hydroxide (*ca.* 20ml) over 30 min. Undissolved ester was filtered off and the filtrate washed with ethyl acetate (4x250ml). The solvent was then evaporated *in vacuo*, the residue dissolved in 6N

5   hydrochloric acid (100ml) then heated under reflux for 5h. After cooling to RT the precipitated benzoic acid was removed by filtration and the filtrate washed with ethyl acetate (2x100ml). The aqueous portion was then evaporated *in vacuo* to provide the optically pure amino-acid as its dihydrochloride salt (9.48g, 81%).

The dihydrochloride salt was dissolved in water (150ml) and treated with Amberlite

10   IRA-67 ion exchange resin to pH 8. The resin was removed by filtration through celite and the solution evaporated *in vacuo* to provide the free amino-acid as a white solid (6.7g, 100%).

Di-*tert*-butyldicarbonate (13g, 60mmol) was added to a stirred suspension of the amino-acid in a mixture of *tert*-butanol (100ml) and water (50ml) while the pH was

15   maintained at 8.5 by the addition of 1M sodium hydroxide (55ml) over 40min. Water (50ml) was added, the mixture washed with heptane (2x100ml) then acidified to pH 3.5 by the addition of solid potassium hydrogen sulphate. The mixture was then evaporated to dryness and the residue triturated with methanol (3x150ml) to extract the product. Evaporation of the solvent *in vacuo* provided the title compound

20   as a white solid which was recrystallised from aqueous ethanol to optical purity (3.27g, 61%).

$[a]_D$ +24.3° (c=1, Trifluoroacetic acid)

Similarly prepared were:

Intermediate 77   (*S*)-(1,1-Dimethylethoxy)carbonyl-[b-(3-pyridyl)]alanine

25   From intermediate 74 as a white solid

$[a]_D$ +16.1° (c=1, Trifluoroacetic acid)

Intermediate 78   (*S*)-(1,1-Dimethylethoxy)carbonyl-2-methoxyphenylalanine

From intermediate 75 as a white solid (11.1g,97%)

$[a]_D$ -15.2° (c=1, MeOH)

30   Intermediate 79   (*RS*)-*N*-Acetyl-[b-(2-pyridyl)]alanine

A solution of sodium methoxide (65.8g, 1.22mol) in methanol (300ml) was treated portionwise with diethylacetamidomalonate (132.3g, 0.61mol) maintaining a

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temperature of *ca.* 45°C. The mixture was then heated under reflux for 15min. The mixture was cooled to 50°C then treated slowly with a suspension of 2-chloromethylpyridine hydrochloride (100g, 0.61mol), the pink suspension was then heated under reflux for a further 6h. Water (500ml) was added followed by 10M

5   sodium hydroxide (122ml, 1.22mol) and the pH was maintained at *ca.* 11 while heating the mixture at 70°C overnight. The mixture was cooled to RT and the methanol removed *in vacuo*. The aqueous residue was washed with ethyl acetate (2x500ml), then acidified to pH 5 and further washed with ethyl acetate (2x500ml), before evaporating *in vacuo* to provide a semi-solid. The residue was triturated with

10   hot ethanol (500ml) and the sodium chloride removed by filtration. The filtrate was then evaporated *in vacuo* and the residue crystallised from methanol-ethyl acetate to provide the title compound (70g, 66%) as a yellow solid.

TLC R$_f$ 0.5 [*n*-BuOH-AcOH-pyridine-H$_2$O (15-3-10-12)]

Similarly prepared were:

15

<u>Intermediate 80</u>   (*RS*)-*N*-Acetyl-[b-(4-thiazolyl)]alanine

From 4-(chloromethyl)thiazole, as a white foam (6.7g, 73%).

TLC Rf 0.37 (1% AcOH-20% Hexane-EtOAc)

<u>Intermediate 81</u>   (*S*)-(1,1-Dimethylethoxy)carbonyl-[b-(2-pyridyl)]alanine

20   A suspension of intermediate 79 (65g, 0.31mol) in aqueous potassium dihydrogen orthophosphate (10mM, 650ml) was warmed to 40°C and the resulting mixture (pH 4) basified to pH 8 with 10M sodium hydroxide (10ml) forming a solution. Acylase 30,000 (1.3g) was added and the mixture incubated at 40°C overnight. The resulting suspension was cooled to RT, the solid removed by filtration, then the

25   filtrate acidified to pH 1 with 2N hydrochloric acid and washed with ethyl acetate (2x300ml). The aqueous portion was evaporated *in vacuo* to provide a semi-solid which was triturated with hot methanol (300ml) and the solid removed by filtration. To the cooled solution was then added 5M sodium hydroxide to pH 10, followed by di-*tert*-butyldicarbonate (28.6g, 0.131mol), the mixture was maintained at pH 10 by

30   the addition of 5M sodium hydroxide (44ml) over a period of 6h. The methanol was then removed *in vacuo*, the aqueous residue washed with ether (2x500ml) then acidified to pH 3 with 1M potassium hydrogen sulphate. The mixture was extracted

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with ethyl acetate (4x500ml), the combined extracts washed with brine (500ml), dried (MgSO₄) and evaporated *in vacuo* to provide the crude product which was crystallised from ethyl acetate as a white solid (11.6g, 23%).

$[a]_D$ -16.0° (c = 1, MeOH)

Similarly prepared were:

Intermediate 82 (S)-N-(1,1-Dimethylethoxy)carbonyl-[b-(4-thiazolyl)]alanine

From intermediate 80, as a white solid (11g, 94%).

HPLC (Chirex (D)-penicillamine; 2mM CuSO₄; 0.7ml/min; RT=3.25min; 99%ee.

Intermediate 83 (S)-N-(1,1-Dimethylethoxy)carbonyl[b-(2-pyridyl)]alanine
N-methyl amide

DCC (4.07g, 19.7mmol) was added to an ice cooled solution of intermediate 81 (5.0g, 18.8mmol) and N-hydroxysuccinimide (2.27g, 19.7mmol) in dry tetrahydrofuran (200ml). After stirring at RT for 3h the mixture was treated with 40%w/v aqueous methylamine (6.8ml, 79 mmol) and stirred for a further 2h. The precipitated solid was removed by filtration, the filtrate evaporated *in vacuo* and the residue dissolved in dichloromethane (100 ml). The solution was washed sequentially with water (2x100ml), 8% sodium bicarbonate (2x100ml) and brine (100ml), dried (MgSO₄) and evaporated *in vacuo* to provide the crude product.

Purification by column chromatography eluting with 2-5% methanol/dichloromethane provided the title compound (2.7g, 51%) as a red foam.

TLC R$_f$ 0.38 (3% MeOH-CH₂Cl₂)

Similarly prepared were:

Intermediate 84 (S)-N-(1,1-Dimethylethoxy)carbonyl-L-[b-(4-thiazolyl)]alanine
N-methyl amide

From intermediate 82, as a white solid (3.0g, 64%).

TLC R$_f$ 0.46 (5% MeOH-CH₂Cl₂)

Intermediate 85 (R)-N-(1,1-Dimethylethoxy)carbonylpenicillamine N-methyl amide

A solution of (R)-N-(1,1-dimethylethoxy)carbonylpenicillamine (14g, 56.1mmol), N-hydroxybenzotriazole (7.6g, 56.1mmol), methylamine hydrochloride (18.9g, 280mmol), N-methylmorpholine (34ml, 308mmol), 4-dimethylaminopyridine (685mg, 5.6mmol) and EDC (11.8g, 62mmol) in anhydrous dimethylformamide

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(300ml) was stirred at RT overnight. The mixture was poured into 10%w/v citric acid (750ml) and extracted with ether (3x500ml). The combined extracts were then washed with 8% sodium bicarbonate (2x500ml) and brine (500ml), dried (MgSO$_4$) and evaporated *in vacuo* to provide the title compound (12.3g, 83%)

5 TLC R$_f$ 0.47 (50% hexane-EtOAc)

Similarly prepared was:

<u>Intermediate 86</u> (1,1-Dimethylethoxy)carbonyl-5-methyl-L-glutamic acid
N-methyl amide

From (1,1-dimethylethoxy)carbonyl-5-methyl-L-glutamic acid, as a white solid (5.4g,

10 94%).

TLC R$_f$ 0.21 (2% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 87</u> (S)-N$_d$-(1,1-Dimethylethoxy)carbonyl-N$_a$-
(benzyloxy)carbonylornithine N-methyl amide

From (S)-N$_d$-(1,1-dimethylethoxy)carbonyl-N$_a$-(benzyloxy)carbonylornithine (11.5g,

15 31.4mmol), as a white solid (11.0g, 94%).

TLC R$_f$ 0.65 (3% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 88</u> (S)-N-(1,1-Dimethylethoxy)carbonyl-2-methoxyphenylalanine
N-methyl amide

From intermediate 78 (500mg, 1.69mmol), as a white solid (380mg, 73%).

20 TLC R$_f$ 0.55 (5% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 89</u> (S)-N-(1,1-Dimethylethoxy)carbonyl-b-(3-pyridyl)alanine
N-methyl amide

From intermediate 77 (2.0g, 7.5mmol), as a white solid (1.0g, 48%).

TLC R$_f$ 0.42 (5% MeOH-CH$_2$Cl$_2$)

25 <u>Intermediate 90</u> (S)-N-(1,1-Dimethylethoxy)carbonyl-b-(4-pyridyl)alanine
N-methyl amide

From intermediate 76 (2.0g, 7.5mmol), as a white solid (1.7g, 81%).

TLC R$_f$ 0.38 (5% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 91</u> (R)-N-(1,1-Dimethylethoxy)carbonyl-(S-methyl)penicillamine
30 N-methyl amide

A solution of iodomethane (0.59ml, 9.55mmol) in methanol (5ml) was added dropwise at 0°C to a stirred solution of intermediate 85 (500mg, 1.51mmol) in a

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mixture of 2M sodium hydroxide (5ml) and methanol (15ml). The mixture was stirred at RT overnight, then the methanol removed *in vacuo*, the residue diluted with water (50ml) and extracted with ether (3x50ml). The combined extracts were washed with brine, dried (MgSO₄) and evaporated *in vacuo* to provide the title compound as a colourless oil (540mg, 99%).

TLC R$_f$ 0.47 (50%hexane-EtOAc)

Intermediate 92   (R)-N-(1,1-Dimethylethoxy)carbonyl-2,2-dimethyl-2-methane sulphonylalanine N-methyl amide

A suspension of Oxone (1.02g, 1.66mmol) in water (5ml) was added to a stirred solution of intermediate 91 (153mg, 0.55mmol) in methanol (5ml) at 0°C, the mixture was allowed to warm to RT and stirred for 4h. The mixture was diluted with water (50ml) and extracted with ethyl acetate (3x50ml). The combined extracts were washed with brine (50ml), dried (MgSO₄) and evaporated *in vacuo* to provide the title compound as a colourless oil (101mg, 59%).

TLC R$_f$ 0.35 (50%hexane-EtOAc)

Intermediate 93   (R)-N-(1,1-Dimethylethoxy)carbonyl-2,2-dimethyl-2-methane sulphinylalanine N-methyl amide

A solution of 3-chloroperbenzoic acid (670mg, 2.89mmol) in dichloromethane (25ml) was ·added to a stirred solution of intermediate 91 (800mg, 2.89mmol) in dichloromethane (25ml) at 0°C, the mixture was allowed to warm to RT and stirred overnight. The mixture was washed sequentially with 10%w/v sodium sulphite (2x50ml), 8% sodium bicarbonate (2x50ml) and brine (50ml), dried (MgSO₄) and evaporated *in vacuo* to provide the crude product as a colourless oil. Purification by flash column chromatography (eluting with 10% methanol-dichloromethane) provided the title compound as a colourless oil (470mg, 55%).

TLC R$_f$ 0.05 (50%hexane-EtOAc)

Intermediate 94   (R)-2,2-Dimethyl-2-methanesulphinylalanine N-methyl amide hydrochloride

A solution of intermediate 93 (470mg, 1.61mmol) in a mixture of dioxane (25ml) and 2M hydrochloric acid (25ml) was stirred at RT overnight. The solvent was

evaporated to dryness *in vacuo* and freeze dried overnight to provide the title compound (370mg, 100%) as a colourless gum.

TLC R_f 0.06 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

Similarly prepared were:

Intermediate 95   (R)-2,2-Dimethyl-2-methanesulphonylalanine N-methyl amide hydrochloride

From intermediate 92 (300mg, 0.97mmol), as a colourless foam (210mg, 100%).

TLC R_f 0.10 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

Intermediate 96   (R)-Penicillamine N-methyl amide hydrochloride

From intermediate 85 (300mg, 1.14mmol), as a colourless foam (230mg, 100%).

TLC R_f 0.13 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

Intermediate 97   (S)-b-(3-Pyridyl)alanine N-methyl amide dihydrochloride

From intermediate 89 (1.2g, 4.29mmol), as a white solid (1.1g, 100%).

TLC R_f 0.05 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

Intermediate 98   (S)-b-(4-Pyridyl)alanine N-methyl amide dihydrochloride

From intermediate 90 (1.6g, 5.73mmol), as a white solid (1.45g, 100%).

TLC R_f 0.08 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

Intermediate 99   (S)-b-(2-Pyridyl)alanine N-methyl amide dihydrochloride

From intermediate 83 (1.4g, 5.01mmol), as a white solid (1.27g, 100%).

TLC R_f 0.13 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

Intermediate 100   (S)-b-(4-Thiazolyl)alanine N-methyl amide hydrochloride

From intermediate 84 (1.0g, 3.5mmol), as a white solid (730mg, 94%).

TLC R_f 0.21 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

Intermediate 101   (R)-(S-Methyl)penicillamine N-methyl amide trifluoroacetate

A solution of intermediate 91 (200mg, 0.72mmol) in dichloromethane (4ml) containing trifluoroacetic acid (2ml) was stirred at RT overnight. The solvent was removed *in vacuo* and any excess trifluoroacetic acid removed by azeotropic distillation with heptane (3x20ml) to provide the title compound (196mg, 94%) as a colourless foam.

TLC R_f 0.32 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

Similarly prepared were:

45

<u>Intermediate 102</u>   (S)-2-(Methoxyphenyl)alanine N-methyl amide trifluoroacetate

From intermediate 88 (200mg, 0.65mmol), as a pale yellow foam (209mg, 100%).

TLC R_f 0.25 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 103</u>   (S)-5-Methyl-glutamic acid N-methyl amide trifluoroacetate

5   From intermediate 86 (707mg, 2.58mmol), as a white solid (728mg, 98%).

TLC R_f 0.37 (1% NEt$_3$-10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 104</u>   (S)-N$_δ$-(1,1-Dimethylethoxy)carbonylornithine N-methyl amide

Intermediate 87 (11g, 29mmol) was hydrogenated at RT and atmospheric pressure

over 10% palladium on carbon (1g) in ethanol overnight. The catalyst was removed

10   by filtration through hyflo and the filtrate evaporated *in vacuo* to provide the title

compound (2.6g, 36%) as a colourless oil.

TLC R_f 0.37 (5% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 105</u>   N-(Phenylmethoxy)carbonyl-(S-methyl)-L-cysteinyl-L-

phenylalanine N-methyl amide

15   EDC (10.5g, 55mmol) was added to a stirred solution of L-phenylalanine N-methyl

amide (8.9g, 50mmol), intermediate 69 (13.8g, 50mmol) and N-

hydroxybenzotriazole (8.1g, 60mmol) in dry tetrahydrofuran (100ml). The mixture

was stirred at RT overnight. The mixture was treated with 1M hydrochloric acid

(300ml) then extracted with ethyl acetate (4x200ml). The combined organic extracts

20   were washed with 8% sodium bicarbonate (2x200ml), water (200ml) and brine

(200ml), dried (MgSO$_4$) and evaporating *in vacuo* to provide the product as a white

solid (16.5g, 75%).

TLC R_f 0.47 (10% MeOH-CHCl$_3$)

<u>Intermediate 106</u>   N-(1,1-Dimethylethoxy)carbonyl-(S)-propylglycinyl-L-

25   phenylalanine N-methyl amide

From intermediate 71 (5g, 23mmol) and L-phenylalanine N-methyl amide (4.1g,

23mmol), as a white solid (7.72g, 89%).

TLC R_f 0.48 (10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 107</u>   N-(1,1-Dimethylethoxy)carbonyl-L-leucyl-L-*tert*-leucine

30   N-methyl amide

From N-(1,1-dimethylethoxy)carbonyl-L-leucine (13.07g, 56mmol) and L-*tert*-leucine

N-methyl amide (8.11g, 56mmol), as a white solid (15.77g, 79%).

TLC R$_f$ 0.25 (5% MeOH-CH$_2$Cl$_2$)

Intermediate 108 N-(1,1-Dimethylethoxy)carbonyl-(S)-(S-methyl)cysteinyl-L-*tert*-leucine N-methyl amide

From intermediate 70 (6.64g, 28mmol) and L-*tert*-leucine N-methyl amide (4.07g, 28mmol), as a white solid (4.26g, 42%).

TLC R$_f$ 0.45 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 109 N-(1,1-Dimethylethoxy)carbonyl-(S)-(O-methyl)serinyl-L-*tert*-leucine N-methyl amide

From intermediate 72 (7.48g, 34mmol) and L-*tert*-leucine N-methyl amide (4.92g, 34mmol), as a white solid (4.26g, 42%).

TLC R$_f$ 0.40 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 110 N-(Phenylmethoxy)carbonyl-L-valinyl-N_δ-(1,1-dimethylethoxy)carbonyl-L-ornithine N-methyl amide

From intermediate 104 (2.6g, 10.6mmol) and N-(Phenylmethoxy)carbonyl-L-valine (2.82g, 11.2mmol), as a white solid (3.0g, 61%).

TLC R$_f$ 0.32 (5% MeOH-CH$_2$Cl$_2$)

Intermediate 111   (S-Methyl)-L-cysteinyl-L-phenylalanine N-methyl amide

A solution of intermediate 105 (2.0g, 4.65mmol) in dichloromethane (10ml) was treated with 25% hydrobromic acid in acetic acid (18.6ml) and the mixture stirred at RT for 1h. Water (10ml) was added and the mixture washed with dichloromethane (3x15ml). The aqueous phase was then basified to pH14 with 5M sodium hydroxide then extracted with dichloromethane (4x30ml). The combined organic extracts were then washed with brine (30ml), dried (MgSO$_4$) and evaporated *in vacuo* to provide the product as a white solid (1.22g, 88%).

TLC R$_f$ 0.31 (10% MeOH-CHCl$_3$)

Intermediate 112 L-Valinyl-N_δ-(1,1-dimethylethoxy)carbonyl-L-ornithine N-methylamide

Intermediate 110 (3.0g, 6.5mmol) was hydrogenated at RT and atmospheric pressure over 10% palladium on carbon (300mg) in ethanol (100ml) overnight. The catalyst

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was removed by filtration through hyflo and the filtrate evaporated *in vacuo* to provide the title compound (2.2g, 99%) as a white solid.

TLC R_f 0.26 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 113   (*S*)-Propylglycinyl-*L*-phenylalanine *N*-methyl amide

A solution of intermediate 106 (5.36g, 14.2mmol) in a mixture of dioxane (250ml) and 2M hydrochloric acid (250ml) was stirred at RT overnight. The solvent was evaporated to dryness *in vacuo* to provide a white solid. The residue was dissolved in water (200ml) washed with dichloromethane (3x100ml) then basified to pH 10 with 2M sodium hydroxide and extracted with dichloromethane (4x100ml). The combined extracts were washed with brine (100ml), dried (MgSO$_4$) and evaporated *in vacuo* to provide the title compound as a white solid (1.45g, 37%).

TLC R_f 0.29 (10% MeOH-CH$_2$Cl$_2$)

Similarly prepared were:

Intermediate 114   (*S*)-(*S*-Methyl)cysteinyl-*L*-tert-leucine *N*-methyl amide

From intermediate 108, as a white solid (3.5g, 97%).

TLC R_f 0.45 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 115   (*S*)-(*O*-Methyl)serinyl-*L*-tert-leucine *N*-methyl amide

From intermediate 109, as a white solid (2.7g, 98%).

TLC R_f 0.40 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 116   *L-Leucyl-L-tert*-leucine *N*-methyl amide

From intermediate 107, as a white solid (11.2g, 99%).

TLC R_f 0.57 (10% MeOH-CH$_2$Cl$_2$)

Intermediate 117   (*R*)-2-Bromo-5-phthalimidopentanoic acid

A solution of *D*-ornithine hydrochloride (35g, 0.208mol) in water (350ml) was treated with copper (II) sulphate (16.6g, 0.104mol). 5M Potassium hydroxide (*ca.* 40ml) was added to pH 3 then *N*-carboethoxyphthalimide (45.5g, 0.208mol) was added and the pH maintained at 9-10 by the addition of 5M potassium hydroxide (*ca.*55ml). After 2h, 48% hydrobromic acid was added to pH 0.4 (*ca.* 77ml) and any resulting precipitate removed by filtration. The filtrate was cooled to < 5°C, then treated with further hydrobromic acid (152ml) and potassium bromide (59g, 0.5mol). The mixture was then treated dropwise over 45 min with a solution of sodium nitrite

(28.6g, 0.41mol) in water (275ml) whilst maintaining a temperature of <5°C. The mixture was then stirred at <5°C overnight. The resulting precipitate was then removed by filtration, dissolved in ethyl acetate (400ml) and the solution washed with water (2x200ml) and brine (200ml), dried (MgSO$_4$) and evaporated *in vacuo* to provide the title compound (40.6g, 47%) as a cream solid.

TLC R$_f$ 0.80 (10% MeOH-CH$_2$Cl$_2$)

Similarly prepared was:

<u>Intermediate 118</u>   (*S*)-2-Bromo-5-phthalimidopentanoic acid

From L-ornithine hydrochloride (20g, 0.118mol), as a cream solid (22g, 57%).

TLC R$_f$ 0.80 (10% MeOH-CH$_2$Cl$_2$)

<u>Intermediate 119</u>   (*S*)-2-Acetylmercapto-5-phthalimidopentanoic acid

Was prepared by the previously described procedure from intermediate 117 (39.9g, 0.11mol), as a pale orange oil (35.6g, 99%).

TLC R$_f$ 0.24 (50% Heptane-EtOAc)

<u>Intermediate 120</u>   (*R*)-2-Acetylmercapto-5-phthalimidopentanoic acid

Was prepared by the previously described procedure from intermediate 118 (20g, 56mmol), as a pale orange oil (17.7g, 99%).

TLC R$_f$ 0.24 (50% Heptane-EtOAc)

<u>Intermediate 121</u>   (*RS*)- 2-(Acetylmercapto)-5-phthalimidopentanoyl-L-leucine
                                 1,1-dimethylethyl ester

EDC (3.64g, 19mmol) was added to a stirred mixture of L-leucine 1,1-dimethylethyl ester (3.93g, 17.6mmol), *N*-hydroxybenzotriazole (2.62g, 19.4mmol), triethylamine (2.51ml, 18mmol) and intermediate 15 (5.94g, 18.5mmol) in dry tetrahydrofuran (200ml). The mixture was stirred overnight then the solvent removed *in vacuo* and the residue partitioned between water (100ml) and ethyl acetate (100ml). The aqueous portion was then extracted with ethyl acetate (2x50ml), the combined extracts washed with water (2x100ml) and brine (100ml), dried (MgSO$_4$) and evaporated *in vacuo* to a colourless oil.

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Purification by column chromatography eluting with hexane/ethyl acetate (2:1) provided the title compound (6.6g, 77%) as a white solid, a 1:1 mixture of diastereoisomers.

TLC R$_f$ 0.42 (EtOAc/Hexane (1:1))

5   <u>Intermediate 122</u>   (RS)-2-(Acetylmercapto)-5-phthalimidopentanoyl-L-leucine

Trifluoroacetic acid (9.0ml, 115mmol) was added to a stirred solution of intermediate 121 (3.0g, 6.1mmol) in dry dichloromethane (40ml) and the mixture stirred at RT overnight. The mixture was concentrated *in vacuo* and the excess trifluoroacetic acid removed by azeotroping with heptane to provide the title

10   compound (2.48g, 94%) as a colourless foam, a 1:1 mixture of diastereoisomers.

TLC R$_f$ 0.42 (EtOAc/Hexane (3:2))

<u>Intermediate 123</u>   (RS)-2-(Acetylmercapto)-4-succinimidobutanoic acid

Was prepared by the procedure previously described for intermediate 36

TLC R$_f$ 0.38 (HOAc/EtOAc/Hexane (0.1:1:1))

15   <u>Intermediate 124</u>   (S-Methyl)-L-cysteinyl-L-tryptophan N-methyl amide

Was prepared by the procedure previously described for intermediate 111

TLC R$_f$ 0.45 (EtOAc/Hexane (1:1))

<u>Intermediate 125</u>   (R S)-2-[(1,1-Dimethylethyl)mercapto]-3-
phthalimidopropananoic acid

20   Was prepared by the procedure previously described for intermediate 43.

TLC R$_f$ 0.48 (HOAc/EtOAc/Hexane (0.1:1:1))

<u>Example 1</u>   (RS)-N-[2,3-Bis-acetylmercaptopropanoyl]-L-leucyl-L-phenylalanine N
methyl amide

EDC (198 mg) was added to a solution of intermediate 14 (209 mg), L-leucyl-L-

25   phenylalanine N-methyl amide (274 mg), and N-hydroxybenzotriazole hydrate (153 mg) in dry THF (10 ml) at 0°C, and the mixture was stirred at that temperature until TLC analysis (5% MeOH-CH$_2$Cl$_2$) indicated a complete consumption of starting materials (72 h). The solvent was removed by evaporation and the residue was partitioned between 1N hydrochloric acid (35 ml) and ethyl acetate (50 ml). The

30   organic layer was separated, washed with aqueous sodium bicarbonate solution (2 x 200 ml) and brine (2 x 20 ml), then dried (MgSO$_4$) and evaporated *in vacuo* to give the crude product. Purification by flash column chromatography (eluting with

2-5% methanol-dichloromethane) gave the title compound as a colourless solid (264 mg).

TLC R_f 0.25 (2% MeOH-CH$_2$Cl$_2$)

Similarly prepared were:

Example 2   (RS)-N-[(2,4-Bis-acetylmercapto)butanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 15 (870mg, 3.7mmol) and L-leucyl-L-phenylalanine N-methyl amide (1.07g, 3.7mmol), as a white solid (1.4g, 74%).

$C_{24}H_{35}N_3O_5S_2$ [509.7]; [MH$^+$=510]

Example 3   (RS)-N-[(2,5-Bis-acetylmercapto)pentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 16 (906mg, 3.6mmol) and L-leucyl-L-phenylalanine N-methyl amide (1.06g, 3.6mmol), as a white solid (1.5g, 79%).

$C_{25}H_{37}N_3O_5S_2$ [523.7]; [MH$^+$=524]

Example 4   (RS)-N-[(2,6-Bis-acetylmercapto)hexanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 17 (895mg, 3.4mmol) and L-leucyl-L-phenylalanine N-methyl amide (991mg, 3.4mmol), as a white solid (1.4g, 74%).

$C_{26}H_{39}N_3O_5S_2$ [537.75]; [MH$^+$=538]

Example 5   (RS)-N-[2-Acetylmercapto-3-methoxycarbonylpropranoyl]-L-leucyl-L-phenylalanine N-methyl amide

From Intermediate 4 (0.26 g), as a colourless solid (0.33 g).

$C_{23}H_{33}N_3O_6S$ [479.6]; [MH$^+$=480]

Example 6   (RS)-N-[2-Acetylmercapto-4-methoxycarbonylbutanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From Intermediate 5 (0.40 g), as a colourless solid (0.67 g).

$C_{24}H_{35}N_3O_6S$ [493.6]; [MH$^+$=494]

Example 7   (RS)-N-[2-Acetylmercapto-5-methoxycarbonylpentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From Intermediate 6 (0.9 g), as a colourless solid (1.1 g).

$C_{25}H_{37}N_3O_6S$ [507.6]; [MH$^+$=508]

51

Example 8   (*RS*)-*N*-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-
             phenylalanine *N*-methyl amide

From Intermediate 7 (0.95 g), as a colourless solid (1.0 g).

$C_{26}H_{39}N_3O_6S$ [521.6]; [MH$^+$=522]

5   Example 9   (*RS*)-*N*-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-
                  tryptophan *N*-methyl amide

From Intermediate 36 (730mg) and *L*-leucyl-*L*-tryptophan *N*-methyl amide (700mg),
as a pale yellow foam (1.0g, 74%)

$C_{33}H_{39}N_5O_6S$ [633.7]; [MH$^+$=634]

10   Example 10   (*RS*)-*N*-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-
                    tryptophan *N*-methyl amide

From Intermediate 7 (375mg) and *L*-leucyl-*L*-tryptophan *N*-methyl amide (500mg),
as a pale yellow foam (440mg, 52%)

$C_{28}H_{40}N_4O_6S$ [560.7]; [MH$^+$=561]

15   Example 11   (*RS*)-*N*-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-valinyl-*L*-
                    phenylalanine *N*-methyl amide

From Intermediate 36 (232mg) and *L*-valinyl-*L*-phenylalanine *N*-methyl amide
(200mg), as a white solid (230mg, 55%)

$C_{30}H_{36}N_4O_6S$ [580.7]; [MH$^+$=581]

20   Example 12   (*RS*)-*N*-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-valinyl-*L*-
                    phenylalanine *N*-methyl amide

From Intermediate 7 (179mg) and *L*-valinyl-*L*-phenylalanine *N*-methyl amide (200mg),
as a white solid (200mg, 56%)

$C_{25}H_{37}N_3O_6S$ [507.6]; [MH$^+$=508]

25   Example 13   (*RS*)-*N*-[2-Acetylmercapto-2-(3-phthalimidophenyl)acetyl]-*L*-leucyl-*L*-
                    phenylalanine *N*-methyl amide

From Intermediate 37 (690mg) and *L*-leucyl-*L*-phenylalanine *N*-methyl amide
(489mg), as a white solid (927mg, 88%)

$C_{34}H_{36}N_4O_6S$ [628.8]; [MH$^+$=629]

30   Example 14   *N*-[2-(Acetylmercapto)-2-[3-*cis*-phthalimidocyclopentyl]acetyl-*L*-leucyl-
                    L-phenylalanine *N*-methyl amide

From intermediate 38 (140mg, 0.4mmol) and L-leucyl-L-phenylalanine N-methyl amide (117mg, 0.4mmol), as a tan foam (197mg, 79%), a 1:1:1:1 of the four expected diastereoisomers.

$C_{33}H_{40}N_4O_6S$ [620.8]; [MH$^+$=621]

5   Example 15   N-[2-(Acetylmercapto)-2-[3-trans-phthalimidocyclopentyl]acetyl-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 39 (235mg, 0.7mmol) and L-leucyl-L-phenylalanine N-methyl amide (197mg, 0.7mmol), as a brown foam (358mg, 85%), a 1:1:1:1 of the four expected diastereoisomers.

10    $C_{33}H_{40}N_4O_6S$ [620.8]; [MH$^+$=621]

Example 16   (S)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(S)- en-leucine N-methyl amide

From intermediate 119 (13.8g, 43mmol) and intermediate 116 (11.2g, 44mmol), as a white solid (11.6g, 48%).

15    $C_{28}H_{40}N_4O_6S$ [560.7]; [MH$^+$=561]

Example 17   (S)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 119 (10g, 31mmol) and L-leucyl-L-phenylalanine N-methyl amide (9.0g, 31mmol), as a white solid (10.5g, 57%).

20    $C_{31}H_{38}N_4O_6S$ [594.7]; [MH$^+$=595]

Example 18   (R)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 120 (1.0g, 3.1mmol) and L-leucyl-L-phenylalanine N-methyl amide (900mg, 3.1mmol), as a white solid (885mg, 48%).

25    $C_{31}H_{38}N_4O_6S$ [594.7]; [MH$^+$=595]

Example 19   (S)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-tryptophan N-methyl amide

From intermediate 119 (411mg, 1.28mmol) and L-leucyl-L-tryptophan N-methyl amide (423mg, 1.28mmol), as a white solid (330mg, 41%).

30    $C_{33}H_{39}N_5O_6S$ [633.7]; [MH$^+$=634]

53

Example 20   *(RS)*-*N*-[2-(Acetylmercapto)-5-phthalimidopentanoyl]-(*S*-methyl)-*L*-
             cysteinyl-*L*-phenylalanine *N*-methyl amide

From intermediate 36 (326mg, 1mmol) and intermediate 114 (350mg, 1mmol), as
a white solid (380mg, 64%).

5    $C_{29}H_{34}N_4O_6S_2$ [598.7]; [MH$^+$=599]

Example 21   *(RS)*-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-(S)-
             propylglycinyl-L-phenylalanine *N*-methyl amide

From intermediate 113 (145mg, 0.52mmol) and intermediate 36 (168mg, 0.52mmol),
as a white solid (160mg, 53%).

10    $C_{30}H_{36}N_4O_6S$ [580.7]; [MH$^+$=581]

Example 22   *(RS)*-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-valinyl-*N*$_\delta$-(1-
             dimethylethoxy)carbonyl-L-ornithine *N*-methyl amide

From   intermediate 112 (508mg, 1.47mmol) and intermediate 36 (491mg,
1.53mmol), as a white solid (687mg, 72%).

15    $C_{31}H_{45}N_5O_8S$ [647.8]; [MH$^+$=648]

Example 23   *(RS)*-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-valinyl-L-
             ornithine *N*-methyl amide trifluoroacetate

A solution of example 22 (585mg, 0.90mmol) in dichloromethane (20ml) containing
trifluoroacetic acid (2ml) was stirred at RT overnight. The solvent was removed
20 *in vacuo* and any excess trifluoroacetic acid removed by azeotropic distillation with
heptane (3x20ml) to provide the title compound (596mg, 99%) as a off white solid.
$C_{26}H_{38}N_5O_6S$ [548.7]; [MH$^+$=549]

Example 24   *(RS)*-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-valinyl-*N*$_\delta$-
25   (acetyl-L-ornithine *N*-methyl amide

Acetyl chloride (0.024ml, 0.34mmol) was added at 0°C to a stirred solution of
example 23 (205mg, 0.31mmol) and *N*-methylmorpholine (0.134ml, 0.31mmol) in
anhydrous dichloromethane (20ml). The mixture was allowed to warm to RT and
stirred for 1h before diluting with dichloromethane (20ml) and washing sequentially
30 with 2N hydrochloride (20ml), 8% sodium bicarbonate (20ml), water (20ml) and
brine (20ml), dried (MgSO$_4$) and evaporated *in vacuo* to a colourless foam.

Purification by flash column chromatography (eluting with 3-5% methanol-dichloromethane) provided the title compound (30mg, 17%) as a white solid.

$C_{28}H_{41}N_5O_7S$ [590.7]; [MH^+=591]
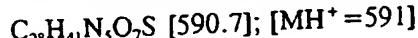
Example 25   (RS)-N-[2-Acetylmercapto-4-phthalimidobutanoyl]-L-leucyl-L-phenylalanine N-methyl amide

A solution of potassium thiolacetate (98 mg) in methanol (2 ml) was added to a suspension of Intermediate 40 (0.5 g) in methanol (10 ml) and the mixture was stirred at RT for 30 min, then at reflux for 6h. The solvent was then removed under reduced pressure and the residue was partitioned between water (50 ml) and dichloromethane (150 ml). The layers were separated and the organic layer was dried over sodium sulfate, filtered, and evaporated to give the crude product. Purification by flash column chromatography (eluting with 50% ethyl acetate-dichloromethane) gave the title compound as a colourless solid (270 mg).

TLC R_f 0.30 (50% EtOAc-CH_2Cl_2)

Similarly prepared were:

Example 26   (RS)-N-[2-Acetylmercapto-5-phthalimidopentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From Intermediate 41 (0.50 g) as a near colourless solid (0.42 g).

TLC R_f 0.20 (50% EtOAc-CH_2Cl_2)

Example 27   (RS)-N-[2-Acetylthio-6-phthalimidohexanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From Intermediate 42 (0.50 g), as a pale yellow solid (0.39 g).

TLC R_f 0.31 (50% EtOAc-CH_2Cl_2)

Example 28   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-[β-(4-thiazolyl)]alanine N-methyl amide

EDC (107mg, 0.56mmol) was added to a stirred solution of intermediate 122 (222mg,0.51 mmol), N-hydroxybenzotriazole 76mg, 0.56mmol), triethylamine (75ml, 0.53mmol) and intermediate 100 (113mg, 0.51mmol) in dry tetrahydrofuran (30ml). The mixture was stirred at RT overnight then the solvent removed *in vacuo* and the residue partitioned between water (20ml) and ethyl acetate (20ml). The aqueous portion was then extracted with ethyl acetate (2x20ml), the combined

extracts washed with water (2x50ml) and brine (50ml), dried (MgSO₄) and evaporated *in vacuo* to a pale yellow oil.

Purification by column chromatography eluting with dichloromethane/methanol (98:2) provided the title compound (170mg, 55%) as a white solid.

5      $C_{28}H_{35}N_5O_6S_2$ [601.7]; [MH⁺=602]

Similarly prepared were:

Example 29   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-ʟ-leucyl-ʟ-[β-(2-pyridyl)]alanine N-methyl amide

From intermediate 122 and intermediate 99, as a white solid (277mg, 67%).

10     $C_{30}H_{37}N_5O_6S$ [595.7]; [MH⁺=596]

Example 30   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-ʟ-leucyl-ʟ-[β-(3-pyridyl)]alanine N-methyl amide

From intermediate 122 and intermediate 97, as a white solid (50mg, 12%).

$C_{30}H_{37}N_5O_6S$ [595.7]; [MH⁺=596]

15   **Example 31**   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-ʟ-leucyl-ʟ-[β-(4-pyridyl)]alanine N-methyl amide

From intermediate 122 and intermediate 98, as a white solid (310mg, 77%).

$C_{30}H_{37}N_5O_6S$ [595.7]; [MH⁺=596]

Example 32   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-ʟ-leucyl-5-methyl-ʟ-glutamic acid N-methyl amide

20

From intermediate 122 and intermediate 103, as a white solid (201mg, 49%).

$C_{28}H_{38}N_4O_8S$ [590.3]; [MH⁺=591]

Example 33   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-ʟ-leucyl-(S)-(2-methoxyphenyl)alanine N-methyl amide

25   From intermediate 122 and intermediate 102, as a white solid (150mg, 37%).

$C_{32}H_{40}N_4O_7S$ [624.8]; [MH⁺=625]

Example 34   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-ʟ-leucyl-(R)-penicillamine N-methyl amide

From intermediate 122 and intermediate 96, as a white solid (230mg, 35%).

30     $C_{27}H_{38}N_4O_6S_2$ [578.8]; [MH⁺=579]

Example 35   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-ʟ-leucyl-(R)-(S-methyl)-penicillamine N-methyl amide

From intermediate 122 and intermediate 101, as a white solid (400mg, 35%).

$C_{28}H_{40}N_4O_6S_2$ [592.8]; [MH$^+$ =593]

Example 36   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(R)-β-dimethyl-2-methanesulphonyl)alanine N-methyl amide

From intermediate 122 and intermediate 95, as a white solid (380mg, 66%).

$C_{28}H_{40}N_4O_8S_2$ [624.8]; [MH$^+$ =625]

Example 37   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(R)-β-dimethyl-2-methanesulphinyl)alanine N-methyl amide

From intermediate 122 and intermediate 94, as a white solid (360mg, 37%).

$C_{28}H_{40}N_4O_7S_2$ [608.8]; [MH$^+$ =609]

Example 38   (RS)-N-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(S)-tert-leucine N-methyl amide

From intermediate 122 and tert-leucine N-methyl amide hydrochloride, as a white solid (120mg, 25%).

$C_{28}H_{40}N_4O_6S$ [560.7]; [MH$^+$ =561]

Example 39   (RS)-N-[2-Mercapto-5-(acetyl)aminopentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

0.4M Sodium hydroxide (0.82ml) was added to a stirred solution of intermediate 57 (203mg, 0.328mmol) and 2-mercaptoethanol (0.23ml, 3.28mmol) in methanol at 0°C. After 15min acetic acid (0.5ml) was added and the solvents evaporated *in vacuo* to provide a yellow oil. Ether (20ml) was added and the resulting precipitate removed by filtration to provide the crude thiol. Purification by flash column chromatography (eluting with 5% methanol in dichloromethane) provided the title compound (86mg, 56%) as a white solid.

$C_{23}H_{36}N_4O_4S$ [464.6]; [MH$^+$ =465]

Similarly prepared were:

Example 40   (RS)-N-[2-Mercapto-5-(benzoyl)aminopentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From intermediate 58 (400mg, 0.59mmol), as a white solid (175mg, 59%).

$C_{28}H_{38}N_4O_4S$ [526.7]; [MH$^+$ =527]

Example 41   (RS)-N-[2-Mercapto-5-(succinimido)pentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

57

From intermediate 59 (600mg, 0.91mmol), as a white solid (147mg, 32%).

$C_{25}H_{36}N_4O_5S$ [504.7]; [MH^+ =505]

Example 42   (RS)-N-[2-Mercapto-5-(methanesulphonyl)aminopentanoyl]-L-leucyl-L-
phenylalanine N-methyl amide

From intermediate 60 (600mg, 1.0mmol), as a white solid (262mg, 57%).

$C_{22}H_{36}N_4O_5S_2$ [500.7]; [MH^+ =501]

Example 43   (RS)-N-[2-Mercapto-5-(benzenesulphonyl)aminopentanoyl]-L-leucyl-L-
phenylalanine N-methyl amide

From intermediate 61 (500mg, 0.7mmol), as a white solid (221mg, 56%).

$C_{27}H_{36}N_4O_5S_2$ [562.8]; [MH^+ =563]

Example 44   (RS)-N-[2-Mercapto-5-(4-pyridylcarbonyl)aminopentanoyl]-L-leucyl-L
phenylalanine N-methyl amide

From intermediate 64 (330mg, 0.48mmol), as a white solid (115mg, 22%).

$C_{27}H_{37}N_5O_4S$ [527.7]; [MH^+ =528]

Example 45   (RS)-N-[2-Mercapto-5-(3-pyridylcarbonyl)aminopentanoyl]-L-leucyl-L
phenylalanine N-methyl amide

From intermediate 65 (200mg, 0.29mmol), as a white solid (34mg, 22%).

$C_{27}H_{37}N_5O_4S$ [527.7]; [MH^+ =528]

Example 46   (RS)-N-[2-Mercapto-5-(2-pyridylcarbonyl)aminopentanoyl]-L-leucyl-L
phenylalanine N-methyl amide

From intermediate 66 (150mg, 0.22mmol), as a white solid (60mg, 52%).

$C_{27}H_{37}N_5O_4S$ [527.7]; [MH^+ =528]

Example 47   (RS)-N-[2-Mercapto-5-(2-pyrazinylcarbonyl)aminopentanoyl]-L-leucyl-
L-phenylalanine N-methyl amide

From intermediate 67 (100mg, 0.15mmol), as a white solid (30mg, 38%).

$C_{26}H_{36}N_6O_4S$ [528.7]; [MH^+ =529]

Example 48   (RS)-N-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-L-[β-(4-
thiazolyl)]alanine N-methyl amide

Concentrated ammonium hydroxide (0.5ml) was added to a solution of example 28
(110mg, 0.18mmol) in methanol (10ml) at 0°C and the mixture was stirred at that
temperature for 3 h. The mixture was diluted with water (10 ml), acidified with 2N
aqueous hydrochloric acid and extracted with dichloromethane (3x20ml). The

combined extracts were dried (Na$_2$SO$_4$) filtered and evaporated to give the crude product. Purification by flash column chromatography (eluting with 2% methanol-dichloromethane) gave the title compound as a colourless solid (71mg, 71%).

C$_{26}$H$_{33}$N$_5$O$_5$S$_2$ [559.7]; [MH$^+$=560]
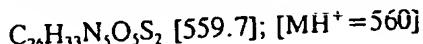
Similarly prepared were:

Example 49   (RS)-N-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-L-[β-(2-pyridyl)]alanine N-methyl amide

From example 29, as a white solid (75mg, 80%).

C$_{28}$H$_{35}$N$_5$O$_5$S [553.7]; [MH$^+$=554]
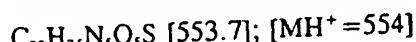
Example 50   (RS)-N-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-5-methyl-L-glutamic acid N-methyl amide

From example 32, as a white solid (68mg, 78%).

C$_{26}$H$_{36}$N$_4$O$_7$S [548.6]; [MH$^+$=549]
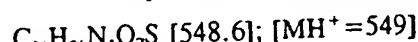
Example 51   (RS)-N-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-(R)-(S-methyl)-penicillamine N-methyl amide

From example 35, as a white solid (160mg, 70%).

C$_{26}$H$_{38}$N$_4$O$_5$S$_2$ [550.8]; [MH$^+$=551]
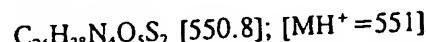
Example 52   (S)-N-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-(S)-tert-leucine N-methyl amide

From example 16, as a white solid (8.12g, 80%).

C$_{26}$H$_{38}$N$_4$O$_5$S [518.7]; [MH$^+$=519]
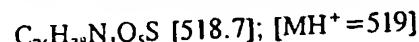
Example 53   (S)-N-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-L-phenylalanine N-methyl amide

From example 17, as a white solid (2.7g, 67%).

C$_{29}$H$_{36}$N$_4$O$_5$S [552.7]; [MH$^+$=553]
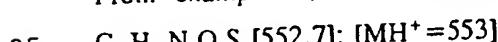
Example 54   (RS)-N-[2,3-Dimercaptopropanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From example 1, as a colourless solid (76 mg).

C$_{19}$H$_{29}$N$_3$O$_3$S$_2$ [411.5]; [MH$^+$=412]
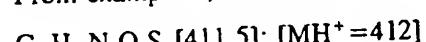
Example 55   (RS)-N-[2-Mercapto-3-methoxycarbonylpropanoyl]-L-leucyl-L-phenylalanine N-methyl amide

From example 5 (0.16 g), as a colourless solid (0.14 g).

59

$C_{21}H_{31}N_3O_5S_2$ [437.5]; [MH$^+$=438]

Example 56   (RS)-N-[2-Mercapto-4-methoxycarbonylbutanoyl]-L-leucyl-L-
                    phenylalanine N-methyl amide

From example 6 (0.18 g), as a colourless solid (0.16 g).

$C_{22}H_{33}N_3O_5S$ [451.5]; [MH$^+$=452]

Example 57   (RS)-N-[2-Mercapto-5-methoxycarbonylpentanoyl]-L-leucyl-L-
                    phenylalanine N-methyl amide

From example 7 (0.32 g), as a colourless solid (0.15 g).

TLC R$_f$ 0.29 (5% MeOH-CH$_2$Cl$_2$)

Example 58   (RS)-N-[2-Mercapto-6-methoxycarbonylhexanoyl]-L-leucyl-L-
                    phenylalanine N-methyl amide

From example 8 (0.31 g), as a colourless solid (0.22 g).

TLC R$_f$ 0.30 (5% MeOH-CH$_2$Cl$_2$)

Example 59   (RS)-N-[2-Mercapto-4-phthalimidobutanoyl]-L-leucyl-L-phenylalanine
                    N-methyl amide

From example 25 (0.20 g), as a pale yellow solid (0.12 g).

$C_{28}H_{34}N_4O_5S$ [538.6]; [MH$_2$$^+$=540]

Example 60   (RS)-N-[2-Mercapto-5-phthalimidopentanoyl]-L-leucyl-L-phenylalanine
                    N-methyl amide

From example 26 (0.2 g), a a colourless solid (0.18 g).

TLC R$_f$ 0.41 (5% MeOH-CH$_2$Cl$_2$)

Example 61   (RS)-N-[2-Mercapto-6-phthalimidohexanoyl]-L-leucyl-L-phenylalanine
                    N-methyl amide

From example 27 (0.15 g), as a pale yellow solid (0.12 g).

TLC R$_f$ 0.25 (10% MeOH-CH$_2$Cl$_2$)

Example 62   (RS)-N-[2-Mercapto-5-phthalimidopentanoyl]-L-leucyl-L-tryptophanN-
                    methyl amide

From example 9 (250mg), as a pale yellow foam (222mg, 96%).

$C_{31}H_{37}N_5O_5S$ [591.7]; [MH$^+$=592]

Example 63   (RS)-N-[2-Mercapto-6-methoxycarbonylhexanoyl]-L-leucyl-L-tryptophan
                    N-methyl amide

From example 10 (330mg), as a colourless foam (300mg, 98%).

$C_{26}H_{38}N_4O_5S$ [518.7]; [MH$^+$ =519]

Example 64 (*S*)-*N*-[2-Mercapto-5-phthalimidopentanoyl]-*L*-(*S*-methyl)cysteinyl-*L*-tryptophan *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 119 and intermediate 124.

$C_{29}H_{32}N_5O_5S_2$ [595.7]; [MH$^+$ =596]

Example 65 (*RS*)-*N*-[2-Mercapto-3-phthalimidopropanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

Was prepared by the procedure described previously for example 39 *via* intermediate 43, from intermediate 125 and *L*-leucyl-*L*-phenylalanine *N*-methyl amide.

$C_{27}H_{32}N_4O_5S$ [524.6]; [MH$^+$ =525]

Example 66 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and *L*-leucyl-*L*-phenylalanine *N*-methyl amide.

$C_{24}H_{34}N_4O_5S$ [490.6]; [MH$^+$ =491]

Example 67 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-(*S*)-propylglycinyl-*L*-phenylalanine *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 113.

$C_{23}H_{31}N_4O_5S$ [475.6]; [MH$^+$ =476]

Example 68 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-*L*-(*S*-methyl)cysteinyl-*L*-*tert*-leucine *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 114.

$C_{19}H_{32}N_4O_5S_2$ [460.6]; [MH$^+$ =461]

Example 69 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-*L*-(*S*-methyl)cysteinyl-*L*-tryptophan *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 124.

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$C_{24}H_{31}N_5O_5S_2$ [533.7]; [MH$^+$=534]

Example 70   (RS)-N-[2-Mercapto-4-succinimidobutanoyl]-L-(S-methyl)cysteinyl-L-phenylalanine N-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 111.

$C_{23}H_{30}N_4O_5S_2$ [506.6]; [MH$^+$=507]

Example 71   (RS)-N-[2-Mercapto-4-succinimidobutanoyl]-L-(O-methyl)serinyl-L-tert-leucine N-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 115.

$C_{19}H_{32}N_4O_5S$ [444.5]; [MH$^+$=445]

Example 72   (RS)-N-[2-Mercapto-4-succinimidobutanoyl]-L-leucyl-L-tert-leucine N-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 116.

$C_{21}H_{36}N_4O_5S$ [456.6]; [MH$^+$=457]

Example 73   (RS)-N-[2-Mercapto-6-carboxyhexanoyl]-L-leucyl-L-tryptophan N-methyl amide

Was prepared by hydrolysis of example 10.

$C_{25}H_{36}N_4O_5S$ [504.7]; [MH$^+$=505]

Example 74   (R)-N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-L-leucyl-L-tryptophan N-methyl amide

Was prepared by separation of the 1:1 mixture of diastereoisomers present in example 10 by flash column chromatography.

$C_{26}H_{38}N_4O_5S$ [518.7]; [MH$^+$=519]

Example 75   (S)-N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-L-leucyl-L-tryptophan N-methyl amide

Was prepared by separation of the 1:1 mixture of diastereoisomers present in example 10 by flash column chromatography.

$C_{26}H_{38}N_4O_5S$ [518.7]; [MH$^+$=519]

Example 76   (R)-N-[2-Acetylmercapto-5-phthalimidopentanoyl]-L-leucyl-L-tryptophan N-methyl amide

Was prepared by separation of the 1:1 mixture of diastereoisomers present in example 9 by flash column chromatography.

$C_{31}H_{37}N_5O_5S_2[591.7]$; [MH$^+$ = 592]

Example 77   (R)-N-[2-Acetylmercapto-5-methoxycarbonylpentanoyl]-L-leucyl-L-phenylalanine N-methyl amide

Was prepared by separation of the 1:1 mixture of diastereoisomers present in example 7 by flash column chromatography.

$C_{25}H_{36}N_4O_5S$ [504.7]; [MH$^+$ = 505]

Example 78   (RS)-N-[2-Mercapto-6-(methylamino)carbonylhexanoyl]-L-leucyl-L-phenylalanine N-methyl amide

Was prepared by hydrolysis of example 8.

$C_{24}H_{38}N_4O_4S$ [478.6]; [MH$^+$ = 479]

Example 79   (RS)-N-[2-Mercapto-6-(amino)carbonylhexanoyl]-L-leucyl-L-phenylalanine N-methyl amide

Was prepared by hydrolysis of example 8.

$C_{23}H_{36}N_4O_4S$ [464.6]; [MH$^+$ = 465]

Example A

Collagenase inhibition activity

The potency of compounds of general formula (I) to act as inhibitors of collagenase was determined by the procedure of Cawston and Barrett, (Anal. Biochem., 99:340-345, 1979) whereby a 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with collagen and collagenase (buffered with 50 mM Tris, pH 7.6 containing 5 mM CaCl$_2$, 0.05% Brij 35, 60 mM NaCl and 0.02% NaN$_3$). The collagen was acetylated ^{3}H or ^{14}C-collagen prepared by the method of Cawston and Murphy (Methods in Enzymolgy, 80:711, 1981). The choice of radiolabel did not alter the ability of collagenase to degrade the collagen substrate. The samples were centrifuged to sediment undigested collagen and an aliquot of the radioactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1mM inhibitor, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the collagenase (IC$_{50}$).

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Example B

Stromelysin inhibition activity

The potency of compounds of general formula (I) to act as inhibitors of stromelysin was determined using the procedure of Nagase et al (Methods in Enzymology Vol

5   254, 1994), whereby a 0.1 mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with stromelysin and ^{3}H transferrin (buffered with 50 mM Tris, pH 7.6 containing 10 mM $CaCl_2$, 150M NaCl, 0.05% Brij, 35, and 0.02% NaN_3). The transferrin was carboxymethylated with ^{3}H iodoacetic acid. The stromelysin activity in the presence of 1 mM, or a dilution

10   thereof, was compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the stromelysin (IC_{50})

Example C

Gelatinase inhibition activity

15   The potency of the compounds of general formula (I) to act as inhibitors of gelatinase was determined using the procedure of Harris & Krane (Biochem Biophys. Acta, 258:566 - 576, 1972), whereby a 1 mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with gelatinase and heat denatured ^{3}H or ^{14}C-acetylated collagen (buffered with 50 mM Tris, pH 7.6

20   containing 5 mM $CaCl_2$, 0.05% Brij 35 and 0.02% NaN_3). The ^{3}H or ^{14}C gelatin was prepared by denaturing ^{3}H or ^{14}C-collagen produced according to the method of Cawston and Murphy (Methods in Enzymology, 80:711, 1981) by incubation at 60°C for 30 minutes. Undigested gelatin was precipitated by addition of trichloroacetic acid and centrifugation. The gelatinase activity in the presence of 1 mM, or dilution

25   thereof, was compared to the activity in a control devoid of inhibitor and results reported as that inhibitor concentration effecting 50% inhibition of the gelatinase (IC_{50}).

Example D

Inhibition of TNFα production

30   The potency of the compounds of general formula (I) to act as inhibitors of the production of TNFa was determined using the following procedure. A 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37° C in an

atmosphere of 5% CO_2 with THP-1 cells (human monocytes) suspended in RPMl 1640 medium and $20\mu M$ β-mercaptoethanol at a cell density of 1 x 10^6/ml and stimulated with $5\mu g$/ml final concentration of LPS. After 18 hours the supernatant is assayed for the levels of TNFα using a commercially available ELISA kit (R & D Systems).

The activity in the presence of 0.1mM inhibitor or dilutions thereof was compared to activity in a control devoid of inhibitor and results reported as that inhibitor concentration effecting 50% inhibition of the production of TNFa.

Example E

Adjuvant arthritic rat model

Compounds of general formula (I) were evaluated in an adjuvant arthritis model in the rat based on the methods employed by B.B. Newbould (1963), Br.J.Pharmacol, 21, 127-136 and C.M. Pearson and F.D. Wood (1959), Arthritis Rheum, 2, 440-459. Briefly male Wistar rats (180-200g) were injected at the base of the tail with Freund's adjuvant. Twelve days later the responding animals were randomised into experimental groups. Compounds of general formula (I) were dosed either orally as a suspension in 1% methyl cellulose or intraperitoneally in 0.2% carboxymethylcellulose from day 12 to the end of the experiment on day 22. Hind paw volumes were measured every two days from day 12 onwards and X-rays were taken of the hind feet on completion of the experiment. Results were expressed as the percent increase of foot volume over day 12 values.

Example F

Mouse ovarian carcinoma xenograft model

Compounds of general formula (I) were evaluated in an ovarian carcinoma xenograft model of cancer, based on that described by B. Davies et al (1993), Cancer Research, 53, 2087-2091 This model, in brief, consists of inoculating female nu/nu mice with 1 x 10^9 OVCAR3-icr cells into the peritoneal cavity. Compounds of general formula (I) are administered by the oral route as a suspension in 1% methyl cellulose or intraperitoneally as a suspension in phosphate buffered saline in 0.01% Tween-20. At the conclusion of the experiment (4-5 weeks) the number of peritoneal cells are counted and any solid tumour deposits weighed. In some

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experiments tumour development is monitored by measurement of tumour specific antigens.

Example G

Rat mammary carcinoma model

5   Compounds of general formula (I) were evaluated in a HOSP.1 rat mammary carcinoma model of cancer (S.Eccles et al (1995), Cancer Research, in press). This model consists of the intravenous inoculation of female CBH/cbi rats with 2 x 10^4 tumour cells into the jugular vein. Compounds of general formula (I) are administered by the oral route as a suspension in 1% methyl cellulose or

10   intraperitoneally as a suspension in phosphate buffered saline in 0.01% Tween-20. At the conclusion of the experiment (4-5 weeks) the animals were killed, the lungs were removed and individual tumours counted after 20 hours fixation in Methacarn.

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CLAIMS

1. A compound of general formula (I):

(I)

wherein:

R^1 is C_{1-6} alkyl, C_{2-6} alkenyl, $-C_{1-6}$ alkyl-aryl, aryl, $-C_{1-6}$ alkyl-heteroaryl, heteroaryl or $-C_{1-6}$ alkyl-AR^9 where A represents O, NR^9 or $S(O)_m$ where $m = 0-2$, and R^9 is H, C_{1-4} alkyl, aryl, heteroaryl, $-C_{1-4}$ alkyl-aryl or $-C_{1-4}$ alkyl-heteroaryl; if $A = NR^9$ the groups R^9 may be the same or different;

R^2 is H or C_{1-6} alkyl;

R^3 is $[Alk]_n R^6$ where Alk is C_{1-6} alkyl or C_{2-6} alkenyl and n is zero or 1;

X is NR^4R^5 where either R^4 is hydrogen or C_{1-6} alkyl optionally substituted by amino (NH_2), aryl, arylamino, protected amino, di(C_{1-6} alkyl)amino, mono(C_{1-6} alkyl)amino, CO_2H, protected carboxyl, carbamoyl, mono(C_{1-6} alkyl)carbamoyl or di(C_{1-6} alkyl)carbamoyl, and R^5 is hydrogen or C_{1-6} alkyl; or NR^4R^5 forms a ring such as pyrrolidino, piperidino or morpholino;

R^7 is hydrogen or $R^{10}CO$ where R^{10} is C_{1-4} alkyl, $-C_{1-4}$ alkyl-aryl, $-C_{1-4}$ alkyl-heteroaryl, cyclo(C_{3-6})alkyl, $-C_{1-4}$ alkyl-cyclo(C_{3-6})alkyl, C_{2-6} alkenyl, $-C_{2-6}$ alkenyl-aryl, aryl or heteroaryl;

R^8 is aryl (substituted with R^{11}), heteroaryl (optionally substituted with R^{11}), C_{1-4} alkyl-R^{11}, $-C_{1-4}$ alkyl-aryl (substituted with R^{11}), $-C_{1-4}$ alkyl-heteroaryl (optionally substituted with R^{11}), cyclo(C_{3-6})alkyl (optionally substituted with R^{11}), cyclo(C_{3-6})alkenyl (optionally substituted with R^{11}), $-C_{1-4}$ alkyl-cyclo(C_{3-6})alkyl (optionally substituted with R^{11}), or any of the three groups

or

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where p is 1 or 2 and B and C are independently selected from O, S, $C(R^9)_2$ and NR^9;

10   R^6 is AR^9, cyclo(C_{3-6})alkyl, cyclo(C_{3-6})alkenyl, C_{1-6} alkyl, -C_{1-6} alkoxy-aryl, benzyloxyaryl, aryl, heteroaryl, -C_{1-3} alkyl-heteroaryl, -C_{1-3} alkyl-aryl, -C_{1-6} alkyl-$COOR^9$, -C_{1-6} alkyl-NHR, $CONHR$, $NHCO_2R$, $NHSO_2R$ or $NHCOR$, R being defined as for R^{10};

     R^{11} is SO_2R^{13}, SR^7, SR^9, COR^{13}, $N(R^9)_2$, NR^9R^{12}, OR^9, succinimido or

15 phthalimido;

     R^{12} is H or COR^9, CO_2R^9 (where R^9 is not H), $CONHR^9$ or SO_2R^9 (where R^9 is not H); and

     R^{13} is OH, OC_{1-4} alkyl, O-C_{1-4} alkyl-aryl, $N(R^9)_2$ (in which the R^9s are the same or different), C_{1-4} alkyl, aryl, heteroaryl, -C_{1-4} alkyl-aryl or -C_{1-4} alkyl-

20 heteroaryl;

the compound being in the form of a non-salt, salt, solvate or hydrate.

2. A compound of claim 1, wherein R^1 is C_{1-6} alkyl or C_{1-6} alkyl-AR^9 where A is $S(O)_m$, NR^9 or O and m=0, 1 or 2, and R^9 is H, C_{1-4} alkyl or aryl.

3. A compound of claim 1 or claim 2, wherein R^3 is $[Alk]_m R^6$ where n=0 or 1, Alk is C_{1-6} alkyl and R^6 is C_{1-6} alkyl, -C_{1-3} alkyl-aryl, -C_{1-3} alkyl-heteroaryl or AR^9.

4. A compound of any preceding claim, wherein R^4 is H.

5. A compound of any preceding claim, wherein X is pyrrolidino, piperidino or morpholino.

6. A compound of any preceding claim, wherein R^7 is H or (C_{1-6} alkyl)carbonyl.

7. A compound of any preceding claim, wherein R^8 is C_{1-4} alkyl-R^{11} or cyclo(C_{3-6})alkyl-R^{11}, and R^{11} is COR^{13}, $NR^9 R^{12}$, $N(R^9)_2$, succinimido or phthalimido, R^{12} is COR^9, $CO_2 R^9$ (provided R^9 is not H) or $SO_2 R^9$ (provided R^9 is not H), and R^{13} is OH, OC_{1-4} alkyl or $N(R^9)_2$.

8. A compound of any preceding claim, wherein R^5 is H or C_{1-6} alkyl.

9. A compound of claim 8, wherein:

R^1 is alkyl, alkenyl, alkylaryl, aryl or alkyl-AR^9 and R^9 is alkyl, aryl or heteroaryl;

R^7 is H or $R^{10}CO$ where R^{10} is alkyl, alkylaryl, cycloalkyl, cycloalkylalkyl, alkenyl or alkenylaryl;

R^8 is optionally-substituted aryl, heteroaryl, alkylaryl, cycloalkyl, cycloalkenyl or alkylcycloalkyl, alkyl-R^{11} or any of the said three groups;

R^6 is cycloalkyl, cycloalkenyl, alkyl, benzyl, alkoxybenzyl, benzyloxylbenzyl or 3-indolylmethyl;

R^{11} is SR^7, SR^9, COR^{13}, $N(R^9)_2$, $NR^9 R^{12}$, OR^9, succinimido or phthalimido; and

R^{13} is OH, Oalkyl, Oalkylaryl, $N(R^9)_2$, alkyl, aryl or alkylaryl.

10. A compound of claim 1, selected from

N-[2,3-*bis*-Acetylmercaptopropanoyl]-L-leucyl-L-phenylalanine N-methylamide

N-[2-Acetylmercapto-3-methoxycarbonylpropanoyl]-L-leucyl-L-phenylalanine N-methylamide

N-[2-Acetylmercapto-4-methoxycarbonylbutanoyl]-L-leucyl-L-phenylalanine N-methylamide

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N-[2-Acetylmercapto-5-methoxycarbonylpentanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-
methylamide

N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-
5   methylamide

N-[2-Acetylmercapto-4-phthalimidobutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-
methylamide

N-[2-Acetylmercapto-6-phthalimidohexanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-
10   methylamide

N-[2,3-*bis*-Mercaptopropanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-3-methoxycarbonylpropanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-
methylamide

N-[2-Mercapto-4-methoxycarbonylbutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

15   *N*-[2-Mercapto-5-methoxycarbonylpentanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-
methylamide

N-[2-Mercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-methylamide

N-[2-Mercapto-4-phthalimidobutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-phenylalanine*N*-methylamideand

20   *N*-[2-Mercapto-6-phthalimidohexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide.

11.   A compound of claim 1, selected from

N-[2-Acetylmercapto-5-methoxycarbonylpentanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-
methylamide

N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-
25   methylamide

N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-valinyl-*L*-phenylalanine   *N*-
methylamide

N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-tryptophan   *N*-
methylamide

30   *N*-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-phenylalanine   *N*-
methylamide

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N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-valinyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-tryptophan *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-[β-(4-thiazolyl)]alanine*N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-[β-(2-pyridyl)]alanine *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-5-methyl-*L*-glutamicacid *N*-methylamide

N-[2-Acetylmercapto-6-phthalimidohexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-2-(3-phthalimido)phenylacetyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-5-methoxycarbonylpentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-tryptophan *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-tryptophan *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-[β-(4-thiazolyl)]alanine *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-[β-(2-pyridyl)]alanine *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-5-methyl-*L*-glutamic acid *N*-methylamide and

N-[2-Mercapto-6-phthalimidohexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide.

12.   A compound of any of claims 1 to 9, wherein R^1 is -CH_2SCH_3.

13.   A compound of claim 12, as defined in any of Examples 20, 64, 68, 69 and 70.

14.   A compound of any of claims 1 to 9, wherein R^3 is tert-butyl or -$C(CH_3)_2S(O)_{0-2}CH_3$.

15. A compound of claim 14, as defined in any of Examples 16, 34, 35, 36, 37, 38, 51, 52 and 72.

16. A compound of any preceding claim, in the form of a single enantiomer or diastereomer, or a mixture of such isomers.

17. A pharmaceutical composition for use in therapy, comprising a compound of any preceding claim, and a pharmaceutically-acceptable diluent or carrier.

18. Use of a compound of any of claims 1 to 16, for the manufacture of a medicament for the treatment or prevention of a condition associated with matrix metalloproteinases or that is mediated by TNFα.

19. Use according to claim 18, wherein the condition is selected from cancer, inflammation and inflammatory diseases, tissue degeneration, periodontal disease, ophthalmological disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infection, HIV infection, shock states, graft versus host reactions, autoimmune disease, reperfusion injury, meningitis and migraine.

20. Use according to claim 19, wherein the condition is selected from tumour growth, angiogenesis, tumour invasion and spread, metastases, malignant ascites and malignant pleural effusion.

21. Use according to claim 19, wherein the condition is selected from rheumatoid arthritis, osteoarthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's disease, atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis.

22. Use according to claim 19, wherein the condition is selected from corneal ulceration, retinopathy and surgical wound healing.

23. Use according to claim 19, wherein the condition is selected from psoriasis, atopic dermatitis, chronic ulcers and epidermolysis bullosa.

24. Use according to claim 19, wherein the condition is periodonititis or gingivitis.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C07K5/06   A61K38/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,95 13289 (CHIROSCIENCE LTD.) 18 May 1995 see the whole document ---	1-24
A	PEPTIDE, CHEMISTRY AND BIOLOGY. PROC. 12TH AM. PEPT. SYMP., CAMBRIDGE, MASS., JUNE 12-16, 1991, 1992 ESCOM, LEIDEN, pages 791-792, R G ALMQUIST ET AL. 'Development of peptidomimetic inhibitors of a newly isolated atrial peptide-degrading enzyme' see the whole document ---	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 January 1996

Date of mailing of the international search report

22.02.96

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		Relevant to claim No.
Category *	Citation of document, with indication, where appropriate, of the relevant passages	
A	CHEMICAL ABSTRACTS, vol. 104, no. 9, 3 March 1986 Columbus, Ohio, US; abstract no. 65259b, F FAHRENHOLZ ET AL. 'Photoaffinity labelling of the renal V2 vasopressin receptor. Identification and enrichment of a vasopressin-binding subunit' page 366; see abstract & EUR. J. BIOCHEM., vol. 152, no. 3, 1986 pages 589-595, ---	1
T	JOURNAL OF BIOLOGICAL CHEMISTRY (MICROFILMS), vol. 269, no. 48, 2 December 1994 MD US, pages 30227-30231, N FOTOUHI ET AL. 'Potent peptide inhibitors of stromelysion based on the prodomain region of matrix metalloproteinase' see the whole document and especially the compounds of Table VI -----	1-24

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9513289	18-05-95	AU-B- 8113394	29-05-95